1979

Thermoperiodic and Photoperiodic Interaction in the Annual Cycle of the Male Green Anole, Anolis Carolinensis.

Teresa A. Noeske
Louisiana State University and Agricultural & Mechanical College

NOESKE, TERESA A.

THERMOPERIODIC AND PHOTOPERIODIC INTERACTION IN THE ANNUAL CYCLE OF THE MALE GREEN ANOLE, ANOLIS CAROLINENSIS

The Louisiana State University and Agricultural and Mechanical Col.

PH.D. 1979

PLEASE NOTE:

In all cases this material has been filmed in the best possible way from the available copy. Problems encountered with this document have been identified here with a check mark ✓.

1. Glossy photographs ✓
2. Colored illustrations _____
3. Photographs with dark background _____
4. Illustrations are poor copy _____
5. Print shows through as there is text on both sides of page _____
6. Indistinct, broken or small print on several pages _____ throughout _____
7. Tightly bound copy with print lost in spine _____
8. Computer printout pages with indistinct print _____
9. Page(s) _____ lacking when material received, and not available from school or author _____
10. Page(s) _____ seem to be missing in numbering only as text follows _____
11. Poor carbon copy _____
12. Not original copy, several pages with blurred type _____
13. Appendix pages are poor copy _____
14. Original copy with light type _____
15. Curling and wrinkled pages _____
16. Other _____

Thermoperiodic and Photoperiodic Interaction in the Annual Cycle of the Male Green Anole, *Anolis carolinensis*

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The Department of Zoology
and Physiology

by

Teresa A. Noeske
B.S., Louisiana State University, 1972
M.S., Louisiana State University, 1974
December, 1979

EXAMINATION AND THESIS REPORT

Candidate: Teresa A. Noeske

Major Field: Physiology

Title of Thesis: Thermoperiodic and Photoperiodic Interaction in the Annual Cycle of the Male Green Anole, Anolis carolinensis

Approved:

Albert H. Meier
Major Professor and Chairman

James B. Traynham
Dean of the Graduate School

EXAMINING COMMITTEE:

H H Tramer

D. A. Rossman

Valentine Lance

Thomas Dietz

Date of Examination:

21 November, 1979

ACKNOWLEDGMENTS

I thank Dr. Albert H. Meier for his insight, patience, and guidance throughout this study. I thank Drs. Meier, Thomas Dietz, Douglas Rossman, Henry Werner, Valentine Lance, and Richard Spieler for critical review of the manuscript. Dr. Spieler gave technical and philosophical aid as well as moral support. Mr. Albert Russo and Drs. Albert Fivizzani, Blaine Ferrell, Nelson Horseman, and John Ottenweller also gave technical aid. Dr. Prentiss Schilling and Ms. Deborah Babcock of the Experimental Statistics Department of Louisiana State University kindly aided in statistical analyses.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Male green anoles Anolis carolinensis were subjected to various temperature and photoperiod regimes at three different times of year. The photoperiods used were similar to that in southern Louisiana where the animals were collected at the respective time of year: April LD 13:11, August LD 13.5:10.5, December LD 10.5:13.5. Lizards were held at one of five temperature regimes at each season: WW (constant 30°C), CC (constant 20°C), and a daily 8 h thermoperiod (8 h 30°C, 16 h 20°C) which was given at one of three times of day relative to the photoperiod (W0-8 = heat from 0-8 h after light onset; W8-16 = heat from 8-16 h after light onset; W16-24 = heat from 16-24 h after light onset). Groups of lizards were held on these regimes for 22 days, and then sacrificed at one of six times of day (one group every 4 h for 24 h).

There were differences in the effects of temperature treatments on reproduction, fattening, and weight gain as a function of the temperature treatment and the time of year this treatment was given. A constant warm temperature regime (WW) was less stimulatory than constant cool temperatures (CC) for weight gain, the rate of fat body lipogenesis, and reproductive potential (the testes of WW animals had fewer germ cell rows in the seminiferous tubules and lower testes wet weight); and was more stimulatory for spermiogenesis (higher reproductive stage) at all three times of year.

There were thermosensitive phases for most of the variables examined. Depending on the time of day the lizards received warmer temperature, increased heat could be stimulatory (thermostimulatory

phase) in comparison to heat treatment at other times of day. The thermostimulatory phase for certain processes changed seasonally. Fat stores were higher in lizards held on W8-16 in April and on W0-8 in August. Weight gain was high in lizards held on any thermoperiod in April, but higher in lizards on W8-16 in August, and W16-24 in December. The thermostimulatory phase for spermiogenesis was 16-24 and 0-8 h after light onset in April, but 8-16 h after light onset in December. Reproductive potential (number of germ cell rows in the seminiferous tubules, testes wet weight) was higher in lizards held in W16-24 in April and December, but on W8-16 in August. The effect of a thermoperiod on reproduction, fattening, and weight gain was not always intermediate to the effects of the constant temperature regimes, but could be greater or less than that of CC or WW.

There were diel variations in almost every physiological variable examined. In the thermoperiod groups, the time of day of peak levels for most variables was related to some aspect of the thermoperiod (i.e., so many hours after heat onset), except peak levels of DNA synthesis which were primarily related to the photoperiod. In the constant temperature groups, peak levels could only be correlated with the photoperiod.

Apparently, the annual physiological cycle of the male green anole is under the environmental influence of both photoperiod and thermoperiod. Because of the extensive interactions between these two environmental stimuli, researchers should be aware that placing green anoles, and presumably other ectotherms on conditions of constant temperatures, may not yield natural results.

INTRODUCTION

Rationale

Annual and daily cycles of a wide variety of physiological processes have been noted in many animals (Bünning, 1973; Luce, 1970). Just as there are seasonal physical changes in nature, there are marked seasonal changes in the physiology of temperate vertebrates in such processes as growth, migration, activity, fattening, and reproduction (Prosser and Brown, 1961; Pengelley, 1974). In order for an animal to be integrated with the changing environment, it must anticipate seasonal change (Cloudsley - Thompson, 1960). For example, the reproductive season in many temperate vertebrates, including fishes and reptiles, is discretely limited to a specific time of year, or even to one specific day or hour. Obviously, the majority of individuals of a species must be reproductively ready to mate at that time. Many birds migrate at specific times of year and must build up fat stores over a period of months prior to migration.

In addition to seasonal changes, an animal is physiologically different throughout a day (Colquhoun, 1971). There are daily rhythms at all organizational levels - cellular, tissue, and organismal. Daily rhythms have been found in a wide variety of cellular metabolic processes such as enzyme activity (see Palmer, 1976), RNA and DNA synthesis (see Ruby et al., 1973), lipogenesis (Meier and Burns, 1976; Meier, 1977), and amino acid metabolism (Wurtman et al., 1968; Fuller, 1970). These individual cellular rhythms are synchronized by daily changes in the environment. The photoperiod (daily cycle of light and

dark) is the most constant environmental cue (Cloudsley-Thompson, 1960) and is believed to be the most potent entrainer - via the neuroendocrine system - of a variety of physiological processes, e.g., photosynthesis; cell division; renal excretion of sodium, chloride, ketosteroids (see Palmer, 1976; Bünning, 1973); and DNA synthesis (Burns et al., 1976).

Temperature has been shown to influence physiological processes in temperate vertebrates, particularly ectotherms whose body temperature varies with the environment more than that of endotherms (Hoffmann, 1969a). The acclimation temperature may modify the effect of a photoperiod. For example, in the lizard Anolis carolinensis the length of the light phase of the photoperiod necessary to stimulate testicular recrudescence increases with lower acclimation temperatures (Licht, 1969a).

A thermoperiod (daily cycle of heat and cold), which more closely mimics the natural daily temperature cycle than a constant temperature, can entrain daily rhythms. A daily temperature change of less than $1^{o}C$ is sufficient to entrain the activity rhythm in a lizard (Hoffmann, 1969a,b). However, temperature cycles are thought to be less effective in setting rhythms than a photoperiod (Bünning, 1973). In contrast to a photoperiod, which acts via the neuroendocrine system, the accepted theory is that the thermoperiod acts peripherally by stimulating those processes (catabolic or anabolic) which occur during the time of heat. Since many metabolic processes have daily rhythms, the effect of temperature will depend on the phase of the cellular rhythm at the time heat occurs. For example, if the heat phase occurs only during the peak time of day of lipogenesis in fat stores, then

mean lipogenesis theoretically will increase. Thus, the time of day that a thermoperiod is given may affect fattening and reproduction by acting differently on the cellular processes underlying these processes. Constant warm temperature would increase the rate of both catabolic and anabolic processes, and would be the cumulative effect of thermoperiods given at different times of day.

However, several experiments have obtained data which question the above theory. For example, the effect of a constant temperature is not always cumulative, but may be the same as that of a thermoperiod (Noeske and Meier, 1977). Also, the effects of a particular thermoperiod on gonadal state and fat stores in a lizard change seasonally, even in animals held on the same photoperiod. Therefore, the thermoperiod may have an important role in the seasonal physiological changes in ectotherms.

The present study was undertaken to determine the effects of constant temperatures versus that of a thermoperiod given at different times of day, on the circadian rhythms of lipogenesis and spermatogenesis. Because an animal's physiology may change seasonally, the experiments were repeated at three different times of year.

Experimental animal

The animal used for the present study was the male green anole, Anolis carolinensis. The green anole is a temperate animal and has distinct seasonal cycles of reproductive activity and fattening. There is perhaps more ecological and physiological information on this animal than on any other reptile.

A. carolinensis has a pair of abdominal fat bodies that are the primary depot for fat (other sites being the liver and carcass), and there is a seasonal cycle of lipid deposition and utilization (Dessauer, 1955). In general, fat body weights are highest when reproductive activity is lowest, and vice versa (Licht and Gorman, 1970, 1975; Derickson, 1976). In August, when gonads are regressing, appetite is very high and lipids are stored. Fat body weights peak in October. The lipids are slowly used up over the winter months while the animal is in brumation (winter dormancy in vertebrate ectotherms; Mayhew, 1965) and presumably food is in low supply. At the end of winter, fat body weights (as percent body weight) are 29% of October levels. In the male anole, fat stores continue to decline through the breeding season and are lowest in July when they reach 6% of October levels (Trobec, 1974). The effects, if any, of photoperiod and thermoperiod on the fattening cycle have not yet been elucidated.

The seasonal reproductive cycle of the male green anole is also well defined. Testicular recrudescence begins in late September with the division of spermatogonia to spermatocytes in the seminiferous tubules. Development continues slowly throughout the winter. The testes and epididymis contain spermatozoa by April. Spermatogenesis (development of sperm) and spermiation (release of sperm from the serminiferous tubules) proceed throughout the summer. In late July, the production of spermatocytes ceases. By late August, the testes are completely involuted and contain only spermatogonia (Hamlett, 1952; Dessauer, 1955; Fox and Dessauer, 1958). The effects of photoperiod and constant temperature regimes on reproduction in the male green anole have been rather thoroughly examined (Fox and Dessauer,

1958; Licht, 1967a,b, 1971a). In summary, the testicular cycle is under the influence of both photoperiod and temperature. The lizard is photoperiodic (sensitive to day length) in nature from late June to early October. During this time, reduction of the hours of light below 14 h induces testicular regression. The termination of the photosensitive phase in fall may be due to exposure to day lengths below 12 h. Recrudescence from fall through spring is controlled primarily by temperature. Low temperatures in fall and winter allow the early stages of spermatogenesis and testis enlargement. Higher temperatures in spring elevate body temperature and spermiation occurs.

In the great majority of the laboratory experiments described above, the effects of constant temperatures and a photoperiod on reproductive and fattening indexes were determined. However, the effect of constant temperatures on these variables is often quite different from that of the more natural thermoperiod (Noeske and Meier, 1977). In the few studies on Anolis using a thermoperiod, the photoperiod was also altered from that natural to the season (Licht, 1966, 1967a,b, 1968, 1969a, 1971a,b; Trobec, 1974; Noeske and Meier, 1977) and thus conflicting data have accumulated. For example, the testes of male A. carolinensis held on LD 14:10 (daily regimen of 14 h of light, 10 h of dark) and on constant 32°C in October were five times as heavy as those held on day 20°C, night 32°C (Licht, 1966) , and the investigator concluded that warm temperatures must occur during the hours of light for testicular recrudescence (Licht, 1972). However, in another study (Noeske and Meier, 1977), green anoles held on LD 6:18 and constant 30°C in October had testes less than one-half the weight of testes of lizards receiving 8 h of heat (8 h 30°C, 16 h 20°C)

just before light onset.

In the following studies, the natural photoperiod of the respective season was used, and only the temperature regime was altered in order to delineate the effects of temperature on the reproductive and fattening cycles.

MATERIALS AND METHODS

Identical experiments were run at three different seasons of year: spring (April 7-27), summer (July 22-August 12), and winter (November 18-December 8). Male anoles were caught in the vicinity of Baton Rouge, Louisiana for the April experiment. Lizards were purchased from the LaPlace Snake Farm for both the August and December experiments. Since male anoles must be at least 55 mm in snout-vent length to be sexually mature (Fox and Dessauer, 1957), the smallest anole used in this study was 3.5 g (approximately 57-58 mm -- Dessauer, 1955; Fox and Dessauer, 1957). Lizards were marked by toe-clipping, weighed, and then placed in plastic cages with a sand substrate, 6-7 animals per cage. Daily photoperiod regimes were LD 13:11 in April, LD 13.5:10.5 in August, and LD 10.5:13.5 in December (approximately the photoperiods of southern Louisiana, 30° North latitude, in the respective months). The green anole sleeps in the open on bushes, shrubs, fence-posts, etc., and thus in nature is normally exposed to the full photoperiod (Oliver, 1955). Light was provided by daylight fluorescent bulbs (150-200 lux at cage level). This level of illumination is high enough to satisfy spectral requirements for photoperiodism in A. carolinensis. The sexual response is essentially independent of light intensity above threshold levels (between 5 and 130 lux) (Licht, 1969b).

Five different temperature regimes were used: constant cold (CC= 20°C ± 0.5°C), constant warm (WW = 30°C ± 0.5°C), and an 8 h thermoperiod (8 h at 30° ± 0.5°C, 16 h at 20° ± 0.5°C) given at one of three different times of day relative to the photoperiod; heat onset was at 0

(W0-8), 8 (W8-16), and 16 h (W16-24) after light onset. The lowest temperature at which A. carolinensis normally becomes active in the field and one at which it remains alert and feeds in the laboratory is 20°C (Gordon, 1956; Licht, 1966). The mean preferred temperature of the lizard is 30°C (Licht, 1964). An 8 h daily thermoperiod (8 h at 30°C, 16 h at 20°C) is the minimum thermoperiod necessary to cause testicular development (Licht, 1969a). Temperature was controlled by placing the cages of the constant temperature groups (CC and WW) in incubators; the thermoperiod groups were kept in a walk-in environmental room (Vista Model D06088-CH). Each of the five temperature treatments was given to six groups of lizards (a total of 36-42 lizards per treatment, 180-210 per experiment). Relative humidity was kept between 50-70%.

Lizards were fed crickets at unscheduled times of day and ad libitum so that each animal could determine its own time of day of feeding. Meal feeding at a particular time of day in fish may affect weight gain (Spieler, 1977; Spieler and Noeske, unpublished data), mean levels of prolactin (Spieler et al., 1977b), and mean levels of thyroxine during the light period (Spieler and Noeske, in preparation).

A water dish was supplied in each cage; water was also sprinkled on the sides of the cages. Watering and routine maintenance were done at unscheduled times of day. Repetition of these stimuli at particular times of day may affect growth and fattening in an ectotherm (Horseman et al., 1976). Handling disturbance alone repeated daily for 10 days may cause marked changes in fat body stores in male A. carolinensis (Meier et al., 1973).

Animals were maintained on the experimental regimes for 3 wk. Approximately 17 days at 31°C is required for the complete conversion of spermatogonia to spermatozoa in A. carolinensis (Licht and Pearson, 1969). Therefore, 3 wk is a sufficient experiment length to accomodate large changes in the gonads if the environmental stimuli are adequate.

On day 22 of the experiments, each lizard was injected intraperitoneally with .75 μCi ^{3}H acetate and 3 μCi ^{3}H thymidine in 0.65% saline (injection volume 0.05 ml). Labeled acetate is taken up in those tissues producing and storing fatty acids; labeled thymidine is taken up during DNA replication. Thymidine is along one of the major "scavenging routes" through which deoxy-thymidine triphosphate is produced (Parish, 1972). Incubation time for both labeled substances was 4 h.

The lizards were starved 48 h before initial and final weighings (Windell and Saroken, 1976). Body weight, wet weights of gonads and fat bodies were determined for each animal. The fat bodies and a testis were stored frozen (-20°C) for future lipid analysis (fat bodies) and DNA analysis (testis). The following were determined from the raw data: change in body weight (final body weight - initial body weight), fat body weight expressed as a percent of body weight (fat body wet weight / final body weight x 100), and gonadosomatic index (GSI = wet weight gonads / final body weight x 100). The use of GSI's or wet weights of gonads for comparison between experimental groups may be misleading due to gonadal edema, etc. (Noeske, 1974). Therefore, histological examination was necessary to draw a clear picture of gonadal state. One testis from each animal was fixed in

Bouin's for histological analysis. In the green anole, both testes are similar in weight and state of development (Licht, 1967a).

The paired fat bodies are the primary lipid depot in the green anole and reflect total body lipids (Dessauer, 1955). Lipids were extracted from fat bodies by a modified Folch method (Folch et al., 1957). The frozen fat bodies were homogenized with chloroform : methanol (2 : 1, v/v) for 4 sec with a Brinkman Polytron. The homogenate was filtered, washed with water to remove non-lipid contaminants, and vortexed. The solution was allowed to stand until the phases separated; the upper phase was siphoned off. The interface surface was washed twice (chloroform : methanol : water, 3:48:47 v/v) to remove any upper phase solutes. The remainder (chloroform and lipids) was poured into a weighed scintillation vial, and evaporated to dryness. The pure lipid in the scintillation vial was weighed, then solubilized with 10 ml scintillation cocktail (12 g terphenyl, 1 liter toluene). The vials were kept in the dark at least one week. The samples were counted for tritium on a scintillation counter for 20 min. Counts per minute were corrected for lipid quenching by comparing a 0.4 min external count with a 0.4 min sample count.

The testis used for histological examination was embedded in paraffin, sliced at 7 μ, and stained with Delafield hematoxylin and eosin. Several variables were examined for each sample: most advanced germ cell type, most abundant germ cell type, diameter of seminiferous tubules, and rows of germ cells (excluding spermatozoa attached to Sertoli cells). The presence or absence of spermatozoa was noted in the epididymis, if present in the tissue section. The above variables were stressed by Fox (1958) as indicators of testis stage.

Rows of germ cells and seminiferous tubule diameter were averaged from at least 5 determinations per sample. Data from Dessauer (1955) and Fox (1958) were used to assess the reproductive state in nature at the same season as the experiments. A reproductive stage (Table I) was also estimated for each sample (Fox, 1958; Licht, 1967a; Noeske, 1974). It was of interest for future research to determine which of the various methods best estimated the reproductive state (see Appendix).

Nucleic acids were extracted from one testis per animal using a modified Schneider (1945) method. Each testis was homogenized with 7 ml cold saline (0.65%) in a Brinkman Polytron for 3 sec. A 3.5 ml aliquot was placed in a test tube, brought to a concentration of 5% trichloroacetic acid (TCA) with the addition of 1 ml 40% TCA, vortexed, centrifuged (2500 rpm, 10 min), and decanted. The remaining pellet was dissolved in 1 ml 10% TCA, and lipids were removed by extracting twice with cold ether. This last step was necessary to remove contaminating lipids which were also labeled with tritium. The tubes were then centrifuged, the supernatant was decanted. The pellet was resuspended in 3 ml 0.5 M hot (70°C) perchloric acid (PCA), vortexed, and incubated 15 min at 70°C (after Webb and Lindstrom, 1965), then centrifuged. A 1 ml aliquot was removed from the supernatant, added to a scintillation vial with 10 ml scintillation cocktail (1 liter Triton X-100, 1 liter toluene, 12 g terphenyl), and counted on a scintillation counter for 10 min.

Another 1.5 ml aliquot was removed from the supernatant and placed in a test tube with 3 ml Dische reagent (Dische, 1955) to determine DNA content. The Burton (1955) modification of the diphenylamine reaction was used; acetaldehyde was added to the reagents and the

solution was allowed to stand for about 16 h at 30°C. The samples were read, along with a standard curve (purified salmon sperm DNA used for standards), on a Bausch and Lomb Spectronic 20 at 600 nm. RNA does not contribute to the Burton colorimetric test for DNA, thus it was not necessary to separate the nucleic acids (Shatkin, 1969).

Data were subjected to two-way analyses of variance (ANOVA) by season, and three-factor analyses of variance with season in the model. Differences in time or treatment within a season were determined by Duncan's multiple range test (Duncan's mrt). The within-season tests were run for all five temperature treatments, for the three thermoperiod groups, and for the two constant temperature groups. If the ANOVA for time of day was significant, differences in times of day within a temperature treatment were determined by Student's *t* test. The square root transformation was used prior to analysis of variance for count per minute data for both ^{3}H-acetate and thymidine incorporation in order to normalize their distribution.

RESULTS

Initial Body Weight

There were no differences in initial body weight at any season among treatment groups or with time of day (Tables II, III, IV) ($P > .05$, two-way ANOVA). There was a seasonal difference in initial body weight ($P < .0001$, three-factor ANOVA). December weights (mean $\pm$ standard error = 5.12$\pm$.06 g) were higher than August weights (4.76 $\pm$ .06 g), which were higher than April weights (4.37 $\pm$ .07 g) ($P < .0001$, Student's t test).

Final Body Weight

April. There was a difference in variance of final body weight with temperature treatment ($P < .001$, two-way ANOVA). Weights were lowest in WW ($P < .05$, Duncan's multiple range test) (Table V).

August. There was a difference in variance of body weight with temperature regime ($P < .04$, two-way ANOVA). The final body weights of animals held in CC and W8-16 were higher than those in WW ($P < .05$, Duncan's multiple range test) (Table VI). There was no significant variance of body weight in the thermoperiod groups with time of day ($P < .09$, two-way ANOVA). However, weights of the thermoperiod groups were higher 12 h after heat onset than at heat onset ($P < .05$, Duncan's multiple range test).

December. There was no difference in variance of final weight (Table VII) ($P > .05$, two-way ANOVA).

Final body weight varied with the season ($P < .0001$, three-factor ANOVA). Weights were greater in December (5.55 ± .06 g) than in August (5.07 ± .07 g) and April (4.99 ± .07 g) ($P < .0001$, Student's t test). Considering all three seasons, body weight varied with temperature treatment ($P < .0003$, three-factor ANOVA). Animals held in CC (5.40 ± .08 g) and W16-24 (5.35 ± .08 g) had higher body weights than those in both WW (4.94 ± .09 g) and W0-8 (5.06 ± .09 g); animals in W8-16 (5.28 ± .09 g) had higher weights than those in WW ($P < .02$, Student's t test).

Change in Body Weight (final body weight - initial body weight)

April. Weight change varied with temperature regimen ($P < .0001$, two-way ANOVA). Lizards in WW gained less weight than those in all other groups ($P < .05$, Duncan's multiple range test) (Table VIII).

August. Weight change varied with the temperature regimen ($P < .02$, two-way ANOVA). Animals in CC gained more weight than those in WW, W0-8, and W16-24; animals in W8-16 gained more weight than those in WW ($P < .05$, Duncan's multiple range test) (Table IX).

December. Weight gain varied with the temperature treatment ($P < .05$, two-way ANOVA); animals receiving W16-24 gained more weight than any other treatment group ($P < .05$, Duncan's multiple range test) (Table X).

Considering all three seasons together (three-factor ANOVA), body weight gain varied with season ($P < .0001$), treatment ($P < .0001$), and interaction between season and temperature treatment ($P < .004$). Animals gained more weight in April (.62 ± .04 g) than in December

(.43 ± .04 g) which gained more than in August (.31 ± .04 g) ($P < .03$, Student's *t* test). Overall temperature treatment differences in weight gain included: animals in WW gained less weight than in all other regimes; animals in W16-24 gained more weight than those in W0-8 (Student's *t* test, $P < .02$) (means ± standard error: WW,.23 ± .05 g; CC, .55 ±.05 g; W0-8, .42 ± .05 g; W8-16, .50 ± .05 g; W16-24, .58 ± .05 g).

Gonad Wet Weight

April. Testes wet weights varied with the temperature regimen ($P < .0001$, two-way ANOVA) (Table XI). Animals held in WW had the lowest mean testes weights; weights were higher in animals held in CC and W16-24 than in W0-8 ($P < .05$, Duncan's multiple range test). There was a significant interaction ($P < .04$, two-way ANOVA) between time of day and temperature treatment in the constant temperature groups. CC gonads weighed more 4 h after light onset; WW weights were highest 20 h after light onset ($P < .03$, Student's *t* test).

August. Gonad weight varied with temperature treatment ($P < .0001$, two-way ANOVA). CC groups had heavier testes than all other groups ($P < .05$, Duncan's multiple range test) (Table XII). There was a significant interaction ($P < .02$, two-way ANOVA) between time of day and temperature treatment in the thermoperiod groups. Overall levels for thermoperiod groups were highest 4 h and lowest 16 h after heat onset ($P < .05$, Duncan's multiple range test), although the highest weight (h after heat onset) for animals held in W0-8 was at 12 h, W8-16 at 0 and 4 h, and W16-24 at 4, 12, and 20 h ($P < .05$,

Student's t test).

December. Mean gonad weight varied with the time of day that the heat phase of the thermoperiod occurred ($P < .05$, two-way ANOVA). Animals held in W16-24 had higher gonad weights than those in W0-8 ($P < .05$, Duncan's multiple range test) (Table XIII). There was no significant daily variation in gonad weight in the three thermoperiod groups ($P < .07$, two-way ANOVA), however, mean levels were higher 4 h after heat onset ($P < .05$, Duncan's multiple range test). WW levels were highest 20 h after light onset ($P < .05$, Student's t test).

Mean gonad weight varied with season ($P < .0001$, three-factor ANOVA). April weight (72 ± 1 mg) were higher than December weights (48 ± 1 mg) which were higher than August (23 ± 1 mg) ($P < .0001$, Student's t test). Overall testes weights (taking all three seasons into account) also varied with temperature treatment ($P < .0001$, three-factor ANOVA); CC groups (54 ± 1 mg) had higher weights, WW groups (40 ± 1 mg) had lower weights than all others; W16-24 (49 ± 1 mg) and W8-16 (49 ± 1 mg) weights were higher than W0-8 weights (44 ± 1 mg) ($P < .008$, Student's t test). Considering all three seasons (three factor ANOVA), there were interactions between season and temperature treatment ($P < .001$) and between temperature treatment and time of day ($P < .003$).

Gonadosomatic Index (GSI)

April. GSI varied with the temperature regime ($P < .0001$, two-way ANOVA); WW animals had lower GSIs than those from other

temperature regimes ($P < .05$,Duncan's multiple range test) (Table XIV). There was no significant variation between temperature treatment and time of day ($P < .08$, two-way ANOVA), however, CC GSIs peaked 4 h after light onset and WW at 4 and 20 h after light onset ($P < .05$, Student's t test); mean GSIs of the thermoperiod groups were highest 8 h after heat onset ($P < .05$, Duncan's multiple range test).

August. GSI varied with the temperature regime ($P < .0001$, two-way ANOVA) (Table XV); animals held in CC had higher GSIs than all other groups ($P < .05$, Duncan's multiple range test). There was a significant interaction ($P < .008$, two-way ANOVA) between the thermoperiod regimes and time of day; GSIs were highest 4 h after heat onset ($P < .05$, Duncan's multiple range test).

December. There were daily variations in GSI levels (Table XVI) ($P < .01$, two-way ANOVA). Overall levels for CC and WW were higher 16 h after light onset; the thermoperiod groups had highest mean levels 4 h after heat onset ($P < .05$, Duncan's multiple range test).

There were seasonal variations in GSI ($P < .0001$, three-factor ANOVA). Levels were higher in April ($1.45 \pm .02$) than in December ($.87 \pm .02$) which were higher than in August ($.44 \pm .02$) ($P < .02$, Student's t test). There were differences in temperature treatment over all three seasons ($P < .0001$, three-factor ANOVA). GSIs of CC ($1.01 \pm .02$) groups were higher than all other groups; WW ($.81 \pm .02$) groups had lower GSIs than all other groups (other means: W0-8, $.89 \pm .02$; W8-16, $.93 \pm .02$; W16-24, $.90 \pm .02$) ($P < .02$, Student's t

test). There were also significant interactions between season and temperature treatment ($P < .0001$, three-factor ANOVA) and between temperature treatment and time of day ($P < .003$, three-factor ANOVA).

Germ Cell Rows

April. The average number of germ cell rows in the seminiferous tubules varied with the temperature regime ($P < .0001$, two-way ANOVA). CC animals had more germ cell rows than all other animals, WW animals had fewer rows than all others, and animals held in W16-24 had more rows than those in W0-8 ($P < .05$, Duncan's multiple range test) (Table XVII). In the thermoperiod groups, there were daily rhythms in germ cell rows ($P < .02$, two-way ANOVA). There were more germ cell rows at 12 and 20 h than at 8 and 16 h after heat onset ($P < .05$, Duncan's multiple range test).

August. The number of germ cell rows varied with the temperature regime ($P < .0001$, two-way ANOVA). CC animals had more rows than all others; W8-16 animals had more cell rows than either WW or W0-8 animals ($P < .05$, Duncan's multiple range test) (Table XVIII). There was a significant interaction between temperature regime and time of day ($P < .02$, two-way ANOVA). Thermoperiod groups had more cell rows at 4 h (W8-16, W16-24) or 8 h (W0-8) after heat onset than at other times of day ($P < .05$, Duncan's multiple range test).

December. There was a significant difference in the variation of number of germ cell rows with temperature regime ($P < .05$, two-way ANOVA). Animals in all three thermoperiod groups (W0-8, W8-16, W16-24) had more rows than the WW group ($P < .05$, Duncan's multiple range

test) (Table XIX). There was a significant ($P < .04$, two-way ANOVA) interaction between time of day and temperature regime in the constant temperature groups. WW peak levels occurred 16 h after light onset, CC peak was 4 h after light onset ($P < .06$, Student's t test).

The number of germ cell rows varied with the season ($P < .0001$, three-factor ANOVA). The number of rows was higher in December (10.29 $\pm$ .12) than in April (7.49 $\pm$ .10) which was higher than in August (4.12 $\pm$ .11) ($P < .0001$, Student's t test). Considering all three seasons (three-factor ANOVA), temperature treatment also determined the number of rows ($P < .0001$). WW animals (6.23 $\pm$ .15) had fewer rows than all other groups. CC (7.85 $\pm$ .14) had more rows than W0-8 (7.29 $\pm$ .15) and W16-24 (7.34 $\pm$ .14); W8-16 (7.82 $\pm$ .14) had more rows than W0-8 ($P < .01$, Student's t test). There were significant interactions between season and temperature treatment ($P < .0001$), temperature treatment and time of day ($P < .02$), and season, temperature treatment and time of day ($P < .01$).

Seminiferous Tubule Diameter

April. Tubule diameter varied with the temperature regime ($P < .0001$, two-way ANOVA). Animals in CC, W0-8, and W16-24 had greater tubule diameters than those in WW and W8-16 ($P < .05$, Duncan's multiple range test) (Table XX). There were also daily rhythms in tubule diameter ($P < .04$, two-way ANOVA). Animals in WW had highest levels 16 h after light onset. All three thermoperiod groups had lowest diameters at the time of heat onset, levels were highest 16 h after heat onset ($P < .05$, Duncan's multiple range test).

August. There were differences in the variance of tubule diameter with temperature treatment ($P < .002$, two-way ANOVA). Animals in CC had larger tubule diameters than those in WW, W0-8, and W16-24 ($P < .05$, Duncan's multiple range test) (Table XXI). There was no significant interaction between temperature treatment and time of day ($P < .09$, two-way ANOVA). However, WW diameters were highest at light onset; levels in the thermoperiod groups were highest 12 h after heat onset ($P < .05$, Duncan's multiple range test).

December. There were daily variations in tubule diameter (Table XXII) ($P < .0005$, two-way ANOVA). Diameter was largest 4 h after light onset in CC and WW ($P < .05$, Duncan's multiple range test), and highest 0-4 h after heat onset in the thermoperiod groups ($P < .05$, Duncan's multiple range test).

There were seasonal variations in seminiferous tubule diameter ($P < .0001$, three-factor ANOVA). April diameters (223 $\pm$ 2 μ) were larger than December diameters (167 $\pm$ 2 μ), which were larger than August diameters (148 $\pm$ 2 μ) ($P < .0001$, Student's t test). Average treatment diameters also varied considering all three seasons ($P < .0001$, three-factor ANOVA). Animals in WW (170 $\pm$ 2 μ) had smaller tubule diameters than all other groups; animals in CC (189 $\pm$ 2 μ) and W16-24 (184 $\pm$ 2 μ) had larger diameters than those in W0-8 (180 $\pm$ 2 μ) and W8-16 (178 $\pm$ 2 μ) ($P < .04$, Student's t test). There were significant interactions between season and temperature treatment ($P < .0001$, three-factor ANOVA), season and time of day ($P < .01$, three-factor ANOVA), and between temperature treatment and time of day

($P < .03$, three-factor ANOVA).

Reproductive Stage and Other Histological Variables

April. Reproductive stage varied with the temperature regime ($P < .0001$, two-way ANOVA). Animals held in WW were at higher stages than all other groups; animals held in W0-8 and W16-24 were at higher stages than those in CC and W8-16 ($P < .05$, Duncan's multiple range test) (Table XXIII). There was a daily rhythm of the reproductive stage in CC; the stage was highest 20 h after light onset ($P < .05$, Duncan's multiple range test).

Spermatozoa were the most advanced cell type in the seminiferous tubules of all animals. However, the relative abundance of cell types varied with the treatment. In WW groups, spermatozoa were the most abundant cell type in the tubules; in CC and the three thermoperiod groups, approximately one-third of the animals had a large proportion of spermatids as well as spermatozoa.

August. There was a difference in variance of reproductive stage with temperature treatment ($P < .0001$, two-way ANOVA). Lizards in CC were at a lower reproductive stage than all other groups; animals in WW were at a higher stage than those in W8-16 ($P < .05$, Duncan's multiple range test) (Table XXIV).

The most advanced cell type in the seminiferous tubules of most lizards in the August experiments was spermatozoa. However, in WW groups, spermatogonia were the most advanced cell type (none other being present) in 20% of the animals; spermatogonia were the most advanced cell type in less than 5% of the animals in all other groups.

For the majority of lizards, the most abundant cell type in the tubules was primarily spermatozoa. However, in 30% of the WW animals, spermatogonia were the most abundant cell types. The thermoperiod groups also had a high percentage of animals with spermatogonia as the most abundant cell type (W0-8, 34.5%; W8-16, 22.6%; W16-24, 36.1%); few animals in CC had abundant spermatogonia (5.3%). A few animals in each temperature treatment group had spermatids as the most abundant cell type (CC, 7.9%; WW, 1.7%; W0-8, 3.4%; W8-16, 3.2%; W16-24, 9.7%).

December. Reproductive stage varied with the temperature treatment ($P < .0001$, two-way ANOVA). The reproductive stage of animals in WW was greater than in all other groups; that of animals in CC was less than in all other groups. The testes of animals in W8-16 were at a higher stage than those in W16-24 ($P < .05$, Duncan's multiple range test) (Table XXV). There were daily rhythms in the reproductive stage ($P < .02$, two-way ANOVA). The reproductive stage of WW animals was highest at 4 and 16 h after light onset; that of CC animals was highest at light onset ($P < .05$, Student's t test). The average of the thermoperiod groups was lowest 20 h after heat onset and highest 0 and 16 h after heat onset ($P < .05$, Duncan's multiple range test).

Spermatozoa were the most advanced cell type for each animal in the December treatment groups. Spermatozoa, spermatocytes, and spermatids were the most abundant cells in the following percents for each temperature regime:

WW	12.9%	spermatocytes	83.9%	spermatids	3.2%	spermatozoa
CC	57.4%	"	42.6%	"		
W0-8	36.8%	"	61.7%	"	1.5%	"
W8-16	35.5%	"	64.5%	"		
W16-24	49.3%	"	50.7%	"		

There were seasonal variations in reproductive stage ($P < .0001$, three-factor ANOVA). The average August reproductive stage (7.18 $\pm$ .02) was higher than April (6.67 $\pm$ .02) which was higher than December (4.84 $\pm$.02) ($P < .0001$, Student's t test). Reproductive stage also varied with temperature treatment over all three seasons ($P < .0001$, three-factor ANOVA). CC (5.99 $\pm$ .03) was less than, and WW (6.50 $\pm$ .03) was greater than all other groups; W0-8 (6.27 $\pm$ .03) was greater than W16-24 (6.19 $\pm$ .03) ($P < .0001$, Student's t test; other mean: W8-16, 6.14 $\pm$ .03). Considering all three seasons (three-factor ANOVA), there were significant interactions between season and treatment ($P < .0001$); season and time of day ($P < .02$); and among season, temperature treatment, and time of day ($P < .03$).

Reproductive Index: Germ Cell Rows x Tubule Diameter

April. This reproductive index varied with temperature treatment ($P < .0001$, two-way ANOVA). CC lizards had a higher index than all other groups; WW animals had a lower index than all other groups; animals in W16-24 had a higher index than those in W0-8 and W8-16 ($P < .05$, Duncan's multiple range test) (Table XXVI). There was a bimodal daily variation ($P < .01$, two-way ANOVA) in this reproductive index in the thermoperiod groups, with troughs 0 and 8 h after heat

onset ($P < .05$, Duncan's multiple range test).

August. The index varied with temperature treatment ($P < .0001$, two-way ANOVA). CC animals had a higher index than all other groups; animals in W8-16 had a higher index than those in WW and W0-8 ($P < .05$, Duncan's multiple range test) (Table XXVII). There were significant ($P < .04$, two-way ANOVA) daily variations in this index depending on the temperature treatment. Peak levels were at light onset in CC animals ($P < .05$, Student's t test) and at 8 (W0-8) and 4 h (W8-16, W16-24) after heat onset in the thermoperiod groups ($P < .05$, Duncan's multiple range test).

December. The reproductive index did not vary significantly with the temperature treatment ($P < .06$, two-way ANOVA). However, animals in W8-16 and W16-24 had a higher index than those in WW ($P < .05$, Duncan's multiple range test) (Table XXVIII). In the constant temperature groups, there was no significant interaction between the time of day and temperature regime ($P < .06$, two-way ANOVA). However, highest levels in WW animals were 16 h after light and in CC were 4 h after light onset ($P < .05$, Student's t test); both W0-8 and W8-16 levels were highest at heat onset ($P < .05$, Student's t test).

There were seasonal differences in variance of germ cell rows x tubule diameter (μ) ($P < .0001$, three-factor ANOVA). Animals in April (1680 ± 20) and December (1730 ± 30) had a higher reproductive index than those in August (650 ± 20) ($P < .0001$, Student's t test). There were also overall treatment differences considering all three

seasons ($P < .0001$, three-factor ANOVA). The reproductive index of animals in WW (1080 ± 30) was less than, and CC (1510 ± 30) greater than all other groups; animals in W16-24 (1420 ± 30) had a higher index than those in W0-8 (1350 ± 30) ($P < .04$, Student's t test; W8-16 levels = 1390 ± 30). Considering all three seasons (three-factor ANOVA), there were significant interactions between season and temperature treatment ($P < .0001$), and temperature treatment and time of day ($P < .05$).

Testis DNA Content (µg)

April. The DNA content of the testis varied with the temperature regime ($P < .03$, two-way ANOVA). The testes of animals held in CC and W8-16 had a higher DNA content than those in WW ($P < .05$, Duncan's multiple range test) (Table XXIX).

August. The DNA content of the testes varied with the temperature regime ($P < .0001$, two-way ANOVA). The testes of animals in CC had a higher DNA content than those in all other temperature groups ($P < .05$, Duncan's multiple range test) (Table XXX). There was a significant interaction ($P < .01$, two-way ANOVA) between time of day and the temperature treatment. The amount of DNA was highest at light onset in CC animals ($P < .05$, Student's t test) and at 4 h after heat onset in thermoperiod groups. Lowest levels in thermoperiod groups were at 16 and 20 h after heat onset ($P < .05$, Duncan's multiple range test).

December. There were no variations in DNA content (Table XXXI)

($P > .05$, two-way ANOVA).

There were seasonal differences in testicular DNA content ($P < .0001$, three-factor ANOVA). The testes of animals in April had a higher (439 ± 9 µg) DNA content than those in December (243 ± 9 µg) which were higher than those in August (135 ± 9 µg) ($P < .0001$, Student's t test). The average DNA content for all three seasons varied with the temperature regime ($P < .0001$, three-factor ANOVA). The testes of animals in CC (308 ± 9 µg) had more DNA (per testis) than in all other groups; animals in W16-24 (266 ± 9 µg) and W8-16 (271 ± 9 µg) had more DNA than those in WW (238 ± 9 µg) ($P < .02$, Student's t test; W0-8 levels = 252 ± 9 µg). There was a significant interaction between season and temperature treatment ($P < .002$, three-factor ANOVA).

Thymidine Incorporation into DNA of Testis - Counts Per Minute (CPM)

April. There was no significant interaction between time of day and temperature treatment (Table XXXII) ($P < .09$, two-way ANOVA). However, CPM levels peaked at 16-20 h after light onset in the thermoperiod groups, and 16 h after light onset in the CC group ($P < .05$, Student's t test) (Fig. 1).

August. CPM varied with the temperature treatment ($P < .001$, two-way ANOVA). Animals in WW had a higher rate and W0-8 a lower rate of thymidine incorporation than in all other temperature regimes ($P < .05$, Duncan's multiple range test) (Table XXXIII). There were some daily variations in CPM ($P < .03$, two-way ANOVA). The CPM of

WW testes peaked at light onset ($P < .03$, Student's t test); the CPM of thermoperiod group testes were lowest 20 h after heat onset ($P < .05$, Duncan's multiple range test) (Fig. 2).

December. There was no significant difference in variance ($P < .08$, two-way ANOVA) of CPM in the constant temperature groups. However, CC testes had higher CPM than WW ($P < .05$, Duncan's multiple range test) (Table XXXIV). The phase of the daily rhythm of CPM varied with the temperature regime ($P < .02$, two-way ANOVA). WW levels were higher 0 and 12 h after light onset; CC and W0-8 levels were high at all time of day except at 8 h after light onset; W16-24 levels were highest 0 h after light onset; and W8-16 levels were high at all time of day except at 4 h after light onset ($P < .05$, Student's t test) (Fig. 3). There were no overall correlations with time after heat onset in the thermoperiod groups.

Radioactivity in the DNA fraction of the testis varied seasonally ($P < .0001$, three-factor ANOVA). CPM (0.14 of one testis) were higher in December (814 $\pm$ 30) than in April (699 $\pm$ 33) which were higher than in August (163 $\pm$ 32) ($P < .01$, Student's t test). There was a significant interaction among season, temperature treatment, and time of day ($P < .0009$, three-factor ANOVA).

mg DNA / g Testis Wet Weight

April. There were no variations in mg DNA in the testis / g testis weight between groups (Table XXXV) ($P > .05$, two-way ANOVA).

August. There were significant ($P < .003$, two-way ANOVA) daily

variations in mg DNA / g gonad (Table XXXVI). The constant temperature groups had higher levels 16 h after light onset ($P < .05$, Duncan's multiple range test); W0-8 levels were highest 16 h after light onset (16 h after heat onset); W16-24 levels were highest at light onset (8 h after heat onset) ($P < .05$, Student's t test).

December. There were no variations in mg DNA / g gonad between groups (Table XXXVII) ($P > .05$, Two-way ANOVA).

The mg DNA / g gonad varied with the season ($P < .0001$, three-factor ANOVA). Levels were higher in April (12.5 $\pm$ 0.4) and August animals (12.9 $\pm$ 0.4) than in December animals (10.5 $\pm$ 0.4) ($P < .0004$, Student's t test). There was a significant interaction between season and time of day ($P < .01$, three-factor ANOVA).

Thymidine Incorporation (CPM) / g DNA of Testis

April. There were no differences in variance between groups (Table XXXVIII) ($P > .05$, two-way ANOVA).

August. There was a difference in variance of CPM / g DNA with the temperature treatment ($P < .0001$, two-way ANOVA). Animals held in WW had higher levels than all other groups; animals in W16-24 had higher levels than CC, W0-8, and W8-16 ($P < .05$, Duncan's multiple range test) (Table XXXIX).

December. There were no significant differences in variance of CPM / g DNA ($P < .06$, two-way ANOVA). However, animals in CC had higher CPM / g DNA than those in W16-24 and WW ($P < .05$, Duncan's

multiple range test) (Table XL). There were daily variations in CPM / g DNA ($P < .05$, two-way ANOVA). The levels in the temperature treatment groups collectively peaked at light onset ($P < .05$, Duncan's multiple range test, Student's t test).

There were seasonal differences in variance of DNA CPM / g DNA ($P < .0001$, three-factor ANOVA). Levels were higher in December (24.1 $\pm$ 0.6) than in August (10.1 $\pm$ 0.7) and April (11.3 $\pm$ 0.7) ($P < .0001$, Student's t test). Considering all three seasons (three-factor ANOVA), overall CPM / g DNA varied with temperature treatment ($P < .01$). Animals in WW (17.9 $\pm$ 0.9) had higher CPM / g DNA than all other groups ($P < .06$, Student's t test; CC, 16.0 $\pm$ 0.9; W0-8, 14.7 $\pm$ 0.9; W8-16, 14.4 $\pm$ 0.9; W16-24, 15.0 $\pm$ 0.9). There were interactions between season and temperature regime ($P < .0001$, three-factor ANOVA).

Fat Body Wet Weight

April. Fat body wet weight varied with temperature treatment ($P < .008$, two-way ANOVA). W8-16 weights were higher than all other group ($P < .05$, Duncan's multiple range test) (Table XLI).

August. There was no significant interaction between temperature treatment and time of day in the thermoperiod groups ($P < .07$, two-way ANOVA). However, thermoperiod group weights were highest 8 h after heat onset ($P < .05$, Duncan's multiple range test) (Table XLII).

December. Fat body weight did not vary significantly with temperature treatment in the thermoperiod groups ($P < .07$, two-way

ANOVA). However, animals in W16-24 had higher fat body wet weights than those in W0-8 ($P < .05$, Duncan's multiple range test) (Table XLIII). Levels were higher in the thermoperiod groups at 12 h after heat onset than at 20 h ($P < .05$, Duncan's multiple range test); WW fat body weights peaked at 20 h after light onset, CC weights at 4 h after light onset ($P < .05$, Student's t test).

Fat body wet weights varied with the season ($P < .0001$, three-factor ANOVA). December weights (137 $\pm$ 4 mg) were higher than August weights (65 $\pm$ 4 mg) which were higher than April weights (45 $\pm$ 4 mg) ($P < .0003$, Student's t test).

Fat Body Weight as Percent Body Weight

April. Fat body weight expressed as percent body weight varied with the temperature treatment ($P < .0006$, two-way ANOVA). Animals held on W8-16 had a higher fat percentage than those on other temperature regimes ($P < .05$, Duncan's multiple range test) (Table XLIV).

August. There was no significant interaction between temperature treatment and time of day in the thermoperiod groups (Table XLV) ($P < .07$, two-way ANOVA). However, weights were highest 8 h after heat onset ($P < .05$, Duncan's multiple range test).

December. Fat body weight as percent of body weight did not vary significantly ($P < .08$, two-way ANOVA). However, thermoperiod groups had higher weights 12 h after heat onset than at 16 or 20 h after heat onset ($P < .05$, Duncan's multiple range test); CC levels

peaked at 4 h, and WW levels at 12 and 20 h after light onset ($P < .05$, Student's t test) (Table XLVI).

There were seasonal differences in variance ($P < .0001$, three-factor ANOVA) in fat body weight expressed as percent body weight. December levels (2.44 $\pm$ .06%) were higher than August (1.20 $\pm$ .06%) which were higher than April (.87 $\pm$ .06%) ($P < .0002$, Student's t test).

Fat Body Lipid Weight

April. There were differences in variance of fat body lipid weight with temperature treatment ($P < .007$, two-way ANOVA). Lipid weights were higher in animals held on W8-16 than in all other groups (Table XLVII) ($P < .05$, Duncan's multiple range test).

August. There was a significant interaction ($P < .03$, two-way ANOVA) between treatment and time of day in the thermoperiod groups; levels were highest 8 h after heat onset ($P < .05$, Duncan's multiple range test) (Table XLVIII).

December. There were no differences in lipid weight between groups (Table XLIX) ($P > .05$, two-way ANOVA).

There were seasonal differences in fat body lipid weight ($P < .0001$, three-factor ANOVA). December weights (102 $\pm$ 3 mg) were higher than August weights (53 $\pm$ 3 mg) which were higher than April weights (32 $\pm$ 3 mg) ($P < .0001$, Student's t test).

Percent Lipid Within Fat Bodies

April. Percent lipid did not vary significantly with temperature treatment ($P < .06$, two-way ANOVA). However, animals held on W16-24 had lower percentages than all other groups ($P < .05$, Duncan's multiple range test) (Table L). There were daily variations in percent lipid ($P < .03$, two-way ANOVA). Levels were highest 12 h after light onset in the constant temperature groups, and were higher 20 h after heat onset than at heat onset in the thermoperiod groups ($P < .05$, Duncan's multiple range test).

August. The percent lipid varied with the time of day ($P < .0007$, two-way ANOVA). In constant temperature groups, levels were higher at 0, 4, 8, and 12 h after light onset than at 16 and 20 h. Among thermoperiod groups, the percent lipid was higher at 8 h after heat onset than at 4 h ($P < .05$, Duncan's multiple range test) (Table LI).

December. The percent lipid in thermoperiod groups varied with time of day ($P < .003$, two-way ANOVA). In these three groups, there was a trough 4 h after heat onset ($P < .05$, Duncan's multiple range test) (Table LII).

The percent lipid in lizard fat bodies varied seasonally ($P < .0001$, three-factor ANOVA). December (74.86 $\pm$ .43%) and August (74.20 $\pm$ .53%) levels were higher than April levels (70.04 $\pm$ .49%) ($P < .0001$, Student's t test). There were significant interactions (three-factor ANOVA) between season and time of day ($P < .0001$) and between temperature treatment and time of day ($P < .006$).

Acetate Incorporation into Paired Fat Bodies - Counts Per Minute

April. Radioactivity in the lipid fraction varied with the temperature treatment ($P < .02$, two-way ANOVA) . Animals held in W8-16 had a higher level of lipogenesis than those in WW ($P < .05$, Duncan's multiple range test) (Table LIII). There were also time of day differences in variance within temperature regimes ($P < .01$, two-way ANOVA). CPM were highest 8 h after light onset in CC and WW groups ($P < .05$, Duncan's multiple range test) whereas CPM were low 8 h after light onset in the three thermoperiod groups ($P < .05$, Student's *t* test). Peak levels occurred 16 h after heat onset in W0-8 and W8-16, and at heat onset in W16-24 ($P < .05$, Student's *t* test) (Fig. 4).

August. Temperature treatment had a significant ($P < .04$, two-way ANOVA) effect on variance of lipid CPM. Animals held in W0-8 had higher CPM than those in WW or W16-24 ($P < .05$, Duncan's multiple range test) (Table LIV). There were also time of day differences in variance of CPM ($P < .0001$, two-way ANOVA). CPM for the CC group were highest 20 h after light onset, and for the WW group, 12 h after light onset ($P < .05$, Student's *t* test). The three thermoperiod groups had the highest CPM 16 h after heat onset ($P < .05$, Student's *t* test) (Fig. 5).

December. There were no significant differences in variance of lipid CPM with time of day ($P < .06$, two-way ANOVA). However, levels of the thermoperiod groups were higher 4 h after heat onset ($P < .05$, Duncan's multiple range test) (Table LV, Fig. 6).

There were seasonal differences in variance of radioactivity in the lipid fraction of lizard fat bodies ($P < .0001$, three-factor ANOVA). CPM were higher in April (660 ± 60) and August (600 ± 60) than in December (289 ± 55) ($P < .0001$, Student's t test). Considering all three seasons (three-factor ANOVA) there were mean treatment differences ($P < .003$). Animals held in WW had lower CPM (311 ± 77) than all other groups ($P < .04$, Student's t test; CC, 527 ± 74; W0-8, 613 ± 76; W8-16, 601 ± 76; W16-24, 463 ± 74). There was a significant interaction (three-factor ANOVA) between season and temperature treatment ($P < .05$); season and time of day ($P < .0001$); temperature treatment and time of day ($P < .0009$); and among season, temperature treatment and time of day ($P < .0001$).

Counts Per Minute ^{3}H-Acetate / g Lipid of Paired Fat Bodies

April. There was a significant interaction between time of day and temperature regime in CPM / g lipid in fat bodies ($P < .01$, two-way ANOVA). CPM / g lipid were highest at 8 h after light onset in both constant temperature regimes, CC and WW ($P < .05$, Duncan's multiple range test). Thermoperiod groups levels were highest at heat onset ($P < .05$, Duncan's multiple range test) (Table LVI). No correlation with photoperiod was noted in the thermoperiod groups.

August. There were time of day differences in variance of CPM / g lipid ($P < .05$, two-way ANOVA). Levels in the constant temperature groups were highest 12 h after light onset; levels in the thermoperiod groups were highest 16 h after heat onset ($P < .05$, Duncan's multiple range test) (Table LVII).

December. CPM / g lipid varied with the time of day ($P < .02$, two-way ANOVA). The levels for both constant temperature groups peaked 4 h after light onset whereas thermoperiod group levels peaked 4 h after heat onset ($P < .05$, Duncan's multiple range test) and were not correlated with photoperiod (Table LVIII).

CPM / g lipid varied seasonally ($P < .0001$, three-factor ANOVA). April levels (33,115 $\pm$ 2,705) were higher than August (23,562 $\pm$ 2,744) which were higher than December (3,644 $\pm$ 2,484) ($P < .008$, Student's t test). Considering all three seasons (three-factor ANOVA), there were significant interactions between season and time of day ($P < .006$); temperature treatment and time of day ($P < .0007$); and among season, temperature treatment, and time of day ($P < .005$).

SUMMARY OF RESULTS

There were differences in the effects of temperature treatments on reproduction, fattening, and weight gain as a function of the temperature treatment and the time of year this treatment was given. The following broadly summarizes the results of the temperature treatments in the three months tested (Tables LIX-LXIV, Fig. 7).

The effects of constant temperatures on the physiological variables examined were similar at all times of year (with one exception, rate of DNA replication (Table LXIII). The constant warm temperature regime (WW) was less stimulatory than constant cold (CC) for weight gain, lipogenesis, and reproductive potential (the testes of WW animals had fewer germ cell rows, and lower testes weight); and was more stimulatory for spermiogenesis (higher reproductive stage).

There were thermosensitive phases for most of the variables examined. Depending on the time of day the lizards received warmer temperatures, increased heat could be stimulatory (thermostimulatory phase) or inhibitory (thermoinhibitory phase) in comparison to heat treatment at other times of day. The thermostimulatory phase for certain processes changed seasonally. Lipogenesis was greater in lizards held on W8-16 in April and on W0-8 in August (Table LXIV). Weight gain was high in lizards held on any thermoperiod in April, but higher in lizards on W8-16 in August, and W16-24 in December (Table LX). Reproductive stage (Fox-Licht stage, see Table I) was higher in lizards held on W0-8 and W16-24 in April, but on W8-16 in December (Table LXII). Reproductive potential (number of germ cell

rows, and gonad wet weight) was higher in lizards held on W16-24 in April and December, but on W8-16 in August (Table LXI). The effect of a thermoperiod on reproduction, fattening, and weight gain was not always intermediate between the effects of the constant temperature regimes, but could be greater than or less than that of CC or WW.

There were diel variations in almost every physiological variable examined. In the thermoperiod groups, the time of day of peak levels for most variables was related to some aspect of the thermoperiod (i.e., so many hours after heat onset), except for peak levels of DNA synthesis which were primarily related to the photoperiod. In the constant temperature groups, peak levels could only be correlated with the photoperiod.

DISCUSSION OF INDIVIDUAL VARIABLES

Body Weight

There were no group differences in initial body weight within seasons. However, there were differences among seasons: December initial body weights were higher than both April and August weights. These differences may be explained on the basis of the time of year and age at which the green anole attains sexual maturity. Hatching occurs from July to September and, depending on the time of year hatched, male anoles in Louisiana may be sexually mature by the next spring-summer breeding season (Michael, 1972). Thus, the December samples included lizards at least 1.25 years old, whereas the April and August samples included younger (and less heavy) lizards at least .8 - 1 year old.

The great majority (92%) of the experimental groups in the present study gained weight during the experiments (Tables VIII, IX, X). The green anole's appetite and food consumption are decreased in late fall and winter, even when food is available ad libitum (Dessauer, 1955). However, the lizards gain weight at all times of year in nature, though at a slightly lower rate during the winter dormancy (Gordon, 1956). A. carolinensis have been brought into the laboratory in the fall and held on long photoperiods and high temperatures. A "fall" anole held on a long photoperiod and warm temperature still has a low level of food intake, but metabolic demands have been increased compared with that of lizards held on cooler temperatures. These lizards lose weight, and mortality is high (Fox and Dessauer, 1957; Licht, 1966; Licht and Jones, 1967); any conclusions drawn on the control of reproduction and fattening in unhealthy animals are at best questionable. Perhaps the use of a "natural" photoperiod in the present study prevented such deleterious

effects even with high temperature.

Since there were no within-season differences in initial body weight in the present study, any difference in final body weight is presumed to be due to the experimental regime. The extent of weight gain varied with temperature treatment (Tables VIII, IX, X). Animals in WW gained the least weight in April and August. Several individuals of Urosaurus ornatus exposed continuously to mean preferred temperatures (37.5°C) lost weight and spermatogenesis was disturbed (Licht, 1965). Researchers have maintained the green anole at 31°C for months to determine the effect of this regime on the reproductive system (Licht, 1971a). Unfortunately, very few of the experiments cited in the literature have reported the effect of environmental regime on the body weight of the green anole.

One explanation for the lower weight gain of animals held at a higher temperature is that the rate of metabolism is increased and more energy is used to maintain this level of metabolism, and less energy is available for growth. Thus, animals in CC should gain the most weight, with weight gain of animals receiving the 8 h thermoperiod regimes falling between the two extreme constant temperature. However, in the present study, one thermoperiod group (W0-8) had a final body weight very similar to that of WW animals (although weight gain and final body weight of WW animals were significantly higher than that of W0-8 animals in April (Tables V, VIII). Also, the mean weight gain and final body weight of animals held on another thermoperiod (W16-24) were either similar to or greater than that of CC animals in April and December. Heat from 0-8 h after light onset was least conducive of the thermoperiod regimes to weight gain in August and December, but not in

April.

Although constant heat caused a decrease in weight gain, heat per se is not inhibitory since animals held on a thermoperiod with 8 h of heat (W16-24) grew more than those held on CC with no hours of heat (Tables VIII, X). It appears that cool temperatures at some part of the day (a cold stimulatory phase) are necessary for an increased growth rate. The weight gain of one thermoperiod regime (W0-8) was lower than that of CC and of other thermoperiod regimes. It may be that 0-8 h after light onset is the cold stimulatory phase for body weight gain. Since there were significant differences in growth among the thermoperiod regimes, there was also a time of day (16-24 h after light onset) where heat was stimulatory (thermostimulatory phase) for weight gain. Therefore, not only the temperature (20^{o} versus 30^{o}C), but the time of day that these temperatures are present and the time of year may determine weight gain.

The green anole remains exposed to the environment on bushes or trees during both day and night (Oliver, 1955). Therefore, the animal receives a daily fluctuation in temperature. On sunny days at most times of year, the green anole behaviorally thermoregulates its body temperature higher than ambient; body temperature falls to ambient temperatures after dark (Clark and Kroll, 1974). In the present experiment, lizards which received 8 h of heat immediately after light onset (W0-8), a normally cool time of day in nature, gained the same amount of weight as those that received heat in the "late afternoon and early evening" (W8-16), a normally warm time of day. However, those animals which received heat just before light onset (W16-24), the coolest time of day in nature, gained significantly more (December) or

the same amount of weight (April, August) as the other thermoperiod groups. Heat applied during the last 4 h before light onset is also conducive to weight gain in goldfish Carassius auratus (Spieler et al., 1977a). Interestingly, goldfish have peak thermal preference and thermal resistance to warmer temperatures before light onset (see Reynolds, 1977). Further studies are underway to elucidate the adaptive value of these relationships in goldfish. The critical thermal maximum (a measure of high temperature tolerance) is also highest before light onset in A. carolinensis held on LD 12:12 and 15^{o}:25^{o}C regime with the high temperature coinciding with the light period (Kosh and Hutchison, 1972). However, a 24 h rhythm in preferred temperature has not been found, or tested for in the green anole. Such a diel rhythm of preferred temperature would be a self-selected thermocycle. An animal could behaviorally change the environmental temperature it is exposed to and thus influence a variety of physiological processes such as weight gain, lipogenesis, and spermatogenesis.

There were daily variations in final body weight only in August lizards. Crickets were removed from the individual cages 48 h before the lizards were killed and so the daily variations in final body weight should be due to water uptake and waste excretion. In the constant temperature groups, body weights were highest during the light phase; in thermoperiod groups, overall levels were lowest immediately prior to or at light onset, but were highest 12 h after heat onset. All lizards were kept in an environmental chamber or incubator, both of which had fans to maintain temperatures. The green anole may lose water up to .6% of its initial body weight per hour depending on the convection rate (Claussen, 1967) and water loss may be greater in such

laboratory conditions than in nature. In nature, A. carolinensis is believed to be diurnally active and presumably eats and drinks during the photophase. However, in the present experiment, there was a peak in lizard body weight 12 h after the onset of heat both in light (W0-8, W16-24) and dark (W8-16) which implies that water intake was increased at that time of day. Florida A. carolinensis have a unimodal activity rhythm at all times of year except summer when the rhythm is bimodal: the lizard is active early morning and late afternoon, and inactive at the warmest time of day (midday, early afternoon) (King, 1966). The thermoperiod in the present study may have a similar effect; the lizard is active and drinks water before and after the heat phase regardless of the photoperiod. These results imply that a thermoperiod may influence the daily activity rhythm of the green anole at some times of year.

Seasonal differences in final body weight were similar to those of initial body weight; December weights were heaviest. Although April initial body weights were smallest, these animals grew more during the 3 wk of the experiment than those in August and December. This cannot be totally explained by the fact that smaller lizards grow faster (Michael, 1972), since initial weights of August lizards were also small. The change in body weight in April ($\bar{x}$ .62 g) also cannot be explained due to increased lipid storage, since fat bodies are lowest at this time of year. The increase in gonad weight (.07 g) is not enough to explain the increase in body weight. Therefore, there may be more lean body growth in April than in August and December despite similar experimental conditions at the three months.

Reproductive State - April

April is the beginning of the breeding season for the green anole, and all experimental animals were spermiating. The reproductive potential was higher in CC than in WW animals: gonad wet weight, testis DNA content, seminiferous tubule diameter, and the number of germ cell rows (not including spermatozoa) were higher in CC animals (Tables XI, XVII, XX, XXIX, LIX). Reproductive stage was higher (more advanced) in WW than in CC animals (Table XXIII). According to a previous study (Pearson et al., 1976), warm temperatures accelerate spermiation and decrease the production of early germ cell types in comparison to cold temperatures. However, in the present study, DNA replication (counts per minute of ^{3}H-thymidine incorporation) in the testis was similar in both CC and WW animals. Since DNA synthesis is completed before the onset of meiosis (Lima-de-Faria and Borum, 1962; Monesi, 1962), the 4 h incubation of ^{3}H-thymidine used in the present experiment labelled only spermatogonia and primary spermatocytes (see also Licht and Pearson, 1969). This would imply that in April, with a normal photoperiod, the formation of early germ cells occurs equally at warm and cold temperatures. Since the number of germ cell rows was lower in WW testes, this regime accelerates spermiation over that of lizards in CC.

Most of the reproductive variables (testes wet weight, gonado-somatic index, number of germ cell rows, germ cell rows x seminiferous tubule diameter, DNA content (ug)) of the thermoperiod groups were more like those of animals in CC than those in WW. Therefore, the reproductive potential of the thermoperiod groups was closer to that of CC. However, the reproductive stages of the thermoperiod groups were not always intermediate to those of the constant temperature groups

(Table XXIII). The testes of W16-24 and WO-8 groups were more like (although significantly less than) those of WW; W8-16 testes were at the same stage as CC. Thus, 8 h of warmth at either 0-8 or 16-24 h after light onset increased the rate of spermiogenesis. At this time of year, 8-16 h after light onset is a thermoinhibitory phase for spermiogenesis, whereas 0-8 and 16-24 h after light onset are thermostimulatory phases.

In summary, heat at 0-8 h after light onset increased the rate of spermiogenesis slightly more than at 16-24 h (WO-8 had fewer germ cell rows and lower gonad weight than W16-24). Sixteen hours of cold at any time of day relative to the photoperiod decreased the rate of spermiogenesis (greater accumulation of cell rows).

Since DNA replication (formation of spermatogonia and primary spermatocytes) is not affected by temperature regimes at this time of year (Table XXXII), the difference between temperature treatment groups must be due to differences in spermiogenesis. The rate of spermiogenesis was higher in WW than in any other group. Also, the weight of WW testes (56 $\pm$ 3 mg) was lower than that normal for the time of year (70 mg) (Fox, 1958) which implies that spermiogenesis was proceeding at such a fast rate that the formation of the early germ cells could not keep up with the demand. This observation may explain in part why the testes of green anoles held on WW and a long photoperiod eventually regress. In these instances, the reproductive stage remains high, but the gonad weight and the number of germ cell rows are decreased. On the other hand, maintaining A. carolinensis for 5 months (starting 15 February) at CC produced unusually large, edemic testes with very few sperm (Licht, 1967a). In the present experiment, CC

testes were slightly larger (80 ± 2 mg) than those in the wild population at this time of year. It is important to remember that neither constant 20°C nor 30°C are normal temperature regimes for a temperate animal, and in this case, neither regime duplicates the effects of the natural environment on the reproductive system.

In the present experiment (April), W8-16 probably most closely approximates the natural environmental conditions for A. carolinensis: heat during the afternoon and early evening. It is interesting to note that the testes of animals held on this thermoperiod are similar to those in CC, although the number of germ cell rows in W8-16 is significantly less than that in CC. Obviously, if the natural breeding season extends over a period of 5 months, there must be a balance between spermiogenesis and formation of early germ cells. In this experiment, a thermoperiod (W8-16) is the temperature regime which most closely achieves this balance.

There were time of day differences in many of the reproductive variables in April. The form of these rhythms and the time of day of peak levels were similar for most of these variables. In the thermoperiod groups, the time of day of peak levels was correlated with some aspect of the thermoperiod rather than the photoperiod (GSI, germ cell rows, seminiferous tubule diameter, germ cell rows x tubule diameter). Most of these rhythms peaked during heat or at heat onset. However, the number of germ cell rows was highest 12 and 20 h after heat onset (4 and 12 h after cold onset).

In contrast, the time of day of peak levels of DNA replication (counts per minute, Fig. 1) in the thermoperiod groups and the CC group occurred 16-20 h after light onset. DNA replication has also

been found to be entrained by light in some mammals (Burns et al., 1976). Although CC and WW testes had the same mean counts per minute, the time of day of peak levels differed. DNA replication in CC testes peaked 16 h after light onset; WW levels were highest 8 h after light onset. Thus, an investigator sampling at only one time of day (e.g., 8 or 16 h after light onset) could easily, albeit wrongly, assume differences in mean levels of DNA replication between the two temperature regimes. A better estimate of mean levels may be obtained by sampling at multiple times of day and then taking an average level.

If higher temperature acts directly by stimulating the rate of DNA synthesis, then the amplitude of the peak and the mean levels should be higher in that thermoperiod group (W8-16) in which the warm phase occurs at the time of peak DNA synthesis in CC (12-16 h after light onset). In April, this does not occur. Perhaps the thermoperiod affects DNA synthesis indirectly by eliciting changes in the neuroendocrine systems involved in meiosis.

Reproductive State - December

December is also a time of year of testicular recrudescence in the green anole,but the effects of temperature regimes on reproduction were different from those in April. In December, the number of early germ cell types is increasing dramatically in animals in the wild. There was not so great a disparity between constant temperature regimes as there was in April. In December, the gonads of WW and CC animals were of the same weight and were similar in all reproductive variables but two: reproductive stage and DNA synthesis (Tables XXV, XXXIV). Although the number of germ cell rows was the same in both constant temperature groups, the composition differed. Twice as many animals in

WW than in CC had spermatids as the most abundant cell type. The testes of WW animals were at a higher reproductive stage because of the greater proportion of spermatids. The testes of CC animals had a greater proportion of spermatogonia and primary spermatocytes, and the formation of these cell types was slowed in WW. The sensitivity of DNA replication to temperature in December was different from that in April. CC was more conducive than WW to the formation of early germ cells (DNA replication) (Table XXXIV). According to Licht (1971a), the reproductive system of the green anole is not responsive to day length (photoperiodic) in either April or December. Thus, the difference in temperature response between the two seasons is presumably not the result of different photoperiods. One alternative, barring the influence of unknown environmental stimuli, is that there is an endogenous seasonal change in the sensitivity of the reproductive system to warmth.

The gonad weight of thermoperiod groups did not differ from those of the constant temperature groups, although testes weights were greater in W16-24 than in WO-8 animals. The reproductive stages of the thermoperiod groups fell in the middle of those for CC (lowest stage) and WW (highest stage; spermiation was beginning) (Table XXV). Except for CC, the reproductive stages were higher in this experiment than that normal for December (Licht, 1971a). In nature in southern Louisiana, the normal high and low temperatures at this time of year are 10^{o} and 20^{o}C; even the "cold" temperature used in this experiment is warm for December, perhaps explaining the higher than normal reproductive stages and weights in this experiment. However, there is a daily variation in Louisiana winter temperatures. The most natural

temperature regime used in this study was W8-16 (warmth during the afternoon, cool at night and early morning). The testes of W8-16 animals had a higher percentage of spermatids than spermatocytes, and had a high reproductive stage more like that of animals in WW than in CC. W16-24 animals in contrast, had testes of high weight and equal amount of spermatocytes and spermatids as the most abundant germ cell type, and had a low reproductive stage more like CC than WW. W0-8 animals were intermediate among the thermoperiod groups; testes weight was low, and reproductive stage was moderate. The number of germ cell rows of all thermoperiod groups was higher than that of WW. Thus, at this time of year, 8 h of heat not only gives testes of a higher reproductive stage than CC, but the potential (the number of germ cell rows) is higher than that of WW.

In summary, as in April, 16 h of cold at any time of day relative to the photoperiod increased the potential (number of germ cell rows) of the testes of thermoperiod groups over that of WW. The cold stimulatory phase for early spermatogenesis (higher DNA replication) in December was 16-24 h after light onset. In April, the thermostimulatory phase for spermiogenesis was 0-8 h after light onset; in December, the thermostimulatory phase was 8-16 h after light onset. Not only was there a seasonal change in reproductive sensitivity to constant temperatures (see above), but there was a seasonal shift in the thermostimulatory phase, and perhaps the presence of a cold stimulatory phase.

There were daily rhythms in several reproductive variables. As in April, the peak levels in thermoperiod groups were correlated with some aspect of the thermoperiod (i.e., heat onset) except for DNA

synthesis which was apparently set by the light/dark cycle (Fig. 3). Most of the peaks for gross variables occurred during the heat phase of the thermoperiod or at the onset of heat. DNA synthesis was high at light onset in all three thermoperiods. There were also peak times of day for the reproductive variables in the constant temperature regimes. In most instances, CC levels peaked at 0-4 h after light onset; depending on the reproductive variable, WW levels peaked either at the same time as CC levels or 4-8 h later (reproductive index, number of germ cell rows, reproductive stage). DNA synthesis levels were high in WW animals at 0 and 12 h after light onset and in CC animals at 4, 12, 16, and 20 h after light onset. Because the levels of DNA synthesis in CC animals were high at all times of day but one, the mean levels of the thermoperiod (WO-8) in which the heat phase coincided with the trough in CC animals (4-8 h after light onset) should have been different from the other temperature regimes if the heat phase of the thermoperiod acts directly by increasing the metabolic activities at the time of day it occurs. There was no difference in mean levels of DNA synthesis among the thermoperiod groups, no matter where the heat phase of the thermoperiod occurred relative to the daily rhythm of DNA synthesis in CC animals.

Reproductive State - August

Constant 20° and 30°C had different effects on the reproductive system of the green anole in August. The testes of lizards kept in WW were very small, with few germ cell rows ($\bar{x}$ = 3.3 where 2.0 rows is complete involution), a decreasing seminiferous tubule diameter, and a high reproductive stage (7.4) (Tables XII, XVIII, XXIV). Testes of CC

animals were larger than WW and had fewer germ cell rows than the April high levels, however, they were at reproductive stage 7.0. CC testes were involuting at a slower rate than those of WW animals. Interestingly, DNA replication was higher in WW testes than in CC although the mean DNA replication in August was only 20% of that in December (Table XXXIII). Thus, at this time of year, the CC gonad is immobilized; the cells are maintained, but there is little spermatogenic activity.

Other investigators have found that autoradiographs of cell cultures of testes incubated for 10 h in ^{3}H-thymidine at 32^{o}C showed label primarily in spermatogonia and primary spermatocytes; at 28^{o}C, no label was taken up (Licht and Basu, 1967). Unfortunately, the season and previous acclimation conditions of the animals were not given for the above experiment. However, these results (Licht and Basu, 1967; the present study) question the generalization that low temperatures are more conducive to the formation of early germ cells (Pearson et al., 1976). The effect of a constant temperature on DNA synthesis changed seasonally. In the present study, constant 20^{o}C was conducive to early germ cell formation in April and December, but not in August.

The reproductive state of the thermoperiod groups was more similar to that of WW than CC animals. Eight h of heat, no matter the time of day given, accelerated the regression process. It is important to note that in this experiment, acceleration of regression by warm temperature is not inhibition of spermatogenesis since DNA replication was higher in WW than in CC testes. Rather, acceleration of regression is a stimulation of spermiogenesis. More animals in WO-8 and W16-24 than in WW had involuted testes, and DNA replication in WO-8 testes was only

one-fourth that of WW testes. Thus, the thermostimulatory phase for spermiogenesis was 0-8 h (and to a lesser degree, 16-24 h) after light onset.

One study (Licht, 1971a) noted that constant warm temperature (32°C) slowed the rate of regression more than fluctuating temperatures (20°:30°C), but no attention was given to thermoperiod-photoperiod relations. The results of the present study indicate that a thermoperiod may either slow down or speed up regression depending on the time of day relative to the light-dark cycle that the heat phase occurs.

There were daily rhythms in many of the reproductive variables. Generally, the rhythms of these variables in the thermoperiod groups peaked 4-8 h after heat onset. The DNA rhythm in these groups was not strictly set by the photoperiod; the trough occurred at light onset, but the peak occurred at some time during the heat phase of the thermoperiod. There were few rhythms of reproductive variables in the constant temperature groups; all peaks occurred at light onset. There was no rhythm of DNA synthesis in the testes of CC groups (Fig. 2) which further emphasizes the inactivity of these gonads in August.

Fattening

The temperature regime had less effect on fat body weight, lipid weight, and lipogenesis than on the reproductive variables. WW and CC groups had the same size fat bodies at all times of year (Tables XLI, XLII, XLIII). These results agree with the literature. The fat bodies of animals maintained on 28° or 32°C and a photoperiod normal for the green anole (10-14 h of light) were similar to those of animals in the wild (Dessauer, 1957; Licht and Jones, 1967; Trobec, 1974). However, lizards held on LD 6:18 and 32°C lost fat or fattened less rapidly

than they would in the wild (Licht and Jones, 1967; Noeske and Meier, 1977). Thus, the weight of the fat bodies in lizards held on the range of normal photoperiods does not seem to be affected by temperature regime. However, the weight of the fat bodies is a net index of lipogenesis and lipolysis. The fat body weight will not differ if lipids are stored and used at the same rate. In the present experiment, less fat was incorporated into the fat bodies of WW animals than those held on any other temperature regime (Tables LII, LIV, LV). The incorporation of ^{3}H-acetate into the fat bodies gives an indication of lipogenesis but not of lipolysis. However, a comparison of the fat body wet weight and the rate of lipogenesis may provide information on the rate of lipolysis. Thus, a lower level of lipogenesis combined with similar sized fat bodies may indicate a lower level of lipolysis in WW animals than in CC animals.

The effect of a thermoperiod on fattening depended on the time of day that heat was present relative to the photoperiod, and this effect changed seasonally. As with weight gain, even though lipogenesis was less in WW than CC fat bodies, heat per se was not inhibitory since some thermoperiod groups (W0-8 in August and W8-16 in April) had higher fat body wet weights and rate of lipogenesis than that of CC animals. In December, W16-24 fat body weights were higher than those of W0-8. However, the higher weight may have been due to a difference in tissue water or protein because there was no difference in fat body lipid among temperature groups.

In summary, the thermostimulatory phase for fattening was shifted from 8-16 h after light onset in April (a month when fat body weights are decreasing) to 0-8 h after light onset in August (a month when fat

storage begins).

Although there were few differences in the present experiment in mean fat body weights or lipogenesis as results of temperature treatments, there were differences in these variables with time of day. In contrast to DNA replication in the testes which in April and December were primarily set by the photoperiod, it appears that lipogenesis, like the majority of reproductive variables, was set by the thermoperiod (Figs. 4,5,6). The time of day of peak lipogenesis changed seasonally; in April and December, the peak in the thermoperiod groups occurred approximately 16 h after heat onset, whereas in August, it was 4 h after heat onset. In the constant temperature groups, peak levels of lipogenesis in WW animals were at 8 h (April) and 12 h (August) after light onset; peaks in CC animals were at 8 h (April) and 20 h (August) after light onset. The thermoperiod groups (W0-8 April, W16-24 August) in which heat occurred at the same time of day as the peak lipogenesis in CC groups did not have higher mean levels of lipogenesis than CC, nor were peak levels at the same time of day as that in CC animals.

A diel variation of hepatic lipogenesis has been shown in birds and a fish (Meier and Burns, 1976). In each case, peak lipogenesis occurred during the latter half of the respective animal's active phase and appeared to follow the rhythm of food consumption although the rhythm of lipogenesis persisted in unfed fish. However, in both the above cases, the animals were kept on a constant temperature, thus no correlation with a thermoperiod was possible. Perhaps the rhythm of lipogenesis is set by other environmental cues when the normal cue (some aspect of the thermoperiod) is absent.

Fat body weights and related variables (fat body weight as

percent body weight, fat body lipid weight, percent lipid in fat bodies) were set by the thermoperiod, as was lipogenesis. However, it is curious that the peaks of testis weight were set by the thermoperiod whereas the DNA replication peaks were primarily set by the photoperiod. Many factors could be involved in the daily variation in testis weight, e.g., water, protein, or lipid. However, some reproductive variables which are not directly affected by water changes (e.g., number of germ cell rows) also had daily rhythms correlated with some aspect of the thermoperiod. It is important to note that fat body wet weights were lowest at the same time of day that testes weights peaked. Perhaps active lipid metabolism is necessary for the cell growth that accompanies each phase of spermatogenesis, and the transfer of lipids from the fat bodies to the testes influences the daily rhythm of testes weight. Lipid, particularly that stored in fat bodies, has been noted to play an important part in the reproductive cycle of female lizards. The fat stores are necessary to produce the first clutch of eggs in spring in the lizard Uta stansburiana (Hahn and Tinkle, 1965). In the salamander, Amphiuma means, the annual cycles of fat body and carcass fat are similar in both sexes (Rose, 1967). Rose implied that lipid was necessary to maintain the testes, and that it was more important for reproduction in the male salamander than in the female.

In male lizards, the energy output for reproduction is not nearly as much as that for females. Thus, it has been largely presumed that fat stores in male lizards are primarily for winter energy stores little connected with reproduction. Removal of fat bodies in A. carolinensis did not stop reproduction, however, the liver size increased and may have taken over the function of lipid storage

(Cuellar, 1973). A. trinitatis is one of the few anoles which lacks a distinct annual fat cycle. However, this lizard also lacks a distinct reproductive cycle and may breed year-round (Gorman and Licht, 1975). Histological studies of A. carolinensis testes have shown great quantities of stored lipids, presumably used as an energy source during the process of spermatogenesis (Guraya, 1971). These necessary lipids may be supplied by the fat stores. Many researchers have suggested that fattening in Anolis may occur only after the breeding season. Perhaps the reproductive and fattening cycles are more entwined than previously believed and the testis cycle is also dependent on the fattening cycle. The depletion of body fat stores by summer may promote testis regression because lipid is no longer available as a source of energy.

CONCLUSIONS

An hypothesis consistent with the results of this study is that the annual physiological cycle of A. carolinensis is under the environmental influence of both the photoperiod and thermoperiod. Whereas the photoperiod alone appears to be the primary entrainer of some daily rhythms (e.g., DNA replication), the thermoperiod alone entrains daily rhythms of other physiological factors. Thus, regardless of the location of the heat period relative to the photoperiod, the peaks of some rhythms occurred a specific number of hours after heat application (e.g., lipogenesis, number of germ cell rows).

Even when the photoperiod is the primary environmental entrainer (e.g., in the constant temperature groups), temperature may have an influence. In the present study, the time of day of peak levels of many variables differed between CC and WW groups. In many instances, these peaks were separated by 4-8 h. Possibly, these effects are mediated by temperature-induced changes in the phase relationship of some endogenous hormone(s). For example, in another ectotherm, a fish Fundulus grandis, maintained on LD 12:12, a change of temperature from constant 20°C to constant 28°C phase shifted the peak of the daily prolactin rhythm (Spieler et al., 1978). Daily injections of this hormone have marked effects on fattening and reproduction in many vertebrates depending on the time of day given (see Meier, 1975).

In addition, the photoperiod and thermoperiod may interact in that the photoperiod may set a thermosensitive phase for specific variables (e.g., number of germ cell rows, fat body wet weight), so that heat during particular times of day causes increases (or

decreases) in these variables. The thermostimulatory phase for lipogenesis in fat bodies and DNA replication in testes did not coincide with the time of day of peak levels of these variables in CC animals. Presumably, some effects of the thermoperiod are mediated indirectly via the neuroendocrine system rather than directly by increasing the metabolic rate at a specific tissue.

The thermosensitive phase apparently undergoes seasonal phase shifts in relation to the photoperiod. Such seasonal shifts may have a role in the seasonal physiology of the green anole by preventing seasonally inappropriate physiological postures in response to unusual weather conditions. For example, unseasonally warm temperatures midday in December would not increase body weight gain as the thermostimulatory phase for weight gain is immediately before light onset at this time of year.

Because of the extensive interactions of these two environmental factors, thermoperiod and photoperiod, researchers should be aware that placing green anoles, and presumably other ectotherms, on conditions of constant temperatures may not yield natural results any more than placing the animals on constant light.

TABLE I

Histological stages used to evaluate testis condition

Reproductive Stage[a]	Description	Fox[b] Number	Licht[c] Number
1	Seminiferous tubules completely involuted, only spermatogonia present	VI	1
2	Seminiferous tubules contain primary spermatocytes as well as spermatogonia	I	2
3	Secondary spermatocytes are abundant in seminiferous tubules	II	3
4	Spermatocytes most abundant cell type, very few sperm present in tubules	II-III	4
5	Many spermatids present; rows of sperm around tubule lumen	III	5
6	Many transforming spermatids around almost every lumen, many sperm, spermiation	IV	6
7	Many sperm surrounding tubule lumen, very few late spermatids, fewer cell rows	V	7
8	Seminiferous tubules almost involuted, few spermatozoa present	VI	7

[a]Reproductive stage number (or increments thereof) referred to in this study

[b]from Fox, 1958; Fox and Dessauer, 1958

[c]from Licht, 1967a

TABLE II

Initial body weight of green anole lizards in April prior to placement on experimental temperature and photoperiod regimes. "Hours after light onset" indicates the time of day that groups (individual cages) would be sacrificed 21 days later, and not time of day of initial weighing.

Regime	Hours after light onset 0	4	8	12	16	20	Means
WW[a]	4.07[f] ±.13	4.06 ±.10	4.21 ±.28	4.65 ±.24	4.40 ±.20	4.55 ±.30	4.32 ±.09
CC[b]	4.45 ±.27	4.87 ±.46	4.48 ±.33	4.63 ±.31	4.13 ±.07	4.30 ±.20	4.47 ±.12
W0-8[c]	4.38 ±.30	4.29 ±.31	4.10 ±.26	4.41 ±.35	4.29 ±.26	4.41 ±.37	4.31 ±.12
W8-16[d]	4.09 ±.14	4.49 ±.14	4.30 ±.29	4.24 ±.28	4.47 ±.23	4.30 ±.26	4.31 ±.09
W16-24[e]	4.57 ±.34	4.30 ±.23	4.49 ±.37	4.49 ±.37	4.22 ±.24	4.47 ±.33	4.42 ±.12

[a] WW = constant 30°C

[b] CC = constant 20°C

[c] W0-8 = 30°C from 0-8 h after light onset; 20°C at all other times

[d] W8-16 = 30°C from 8-16 h after light onset; 20°C at all other times

[e] W16-24 = 30°C from 16-24 h after light onset; 20°C at all other times

[f] Mean initial body weight (g) ± standard error of the mean

TABLE III

Initial body weight of green anole lizards in August prior to placement on experimental temperature and photoperiod regimes. "Hours after light onset" indicates the time of day that groups (individual cages) would be sacrificed 21 days later, and not time of day of initial weighing.

Regime	Hours after light onset 0	4	8	12	16	20	Means
WW[a]	4.61[f] ±.30	4.43 ±.27	4.67 ±.29	4.66 ±.41	4.86 ±.32	4.72 ±.22	4.65 ±.12
CC[b]	5.00 ±.45	4.89 ±.35	5.12 ±.39	4.53 ±.25	4.59 ±.40	4.83 ±.34	4.82 ±.14
W0-8[c]	4.76 ±.30	4.77 ±.32	5.15 ±.26	4.38 ±.20	4.60 ±.18	4.76 ±.38	4.72 ±.11
W8-16[d]	4.73 ±.35	4.91 ±.36	4.56 ±.33	4.80 ±.27	4.48 ±.26	5.16 ±.24	4.77 ±.12
W16-24[e]	4.66 ±.25	4.62 ±.24	4.64 ±.29	4.76 ±.43	4.72 ±.27	5.30 ±.53	4.78 ±.14

[a] WW = constant 30°C

[b] CC = constant 20°C

[c] W0-8 = 30°C from 0-8 h after light onset; 20°C at all other times

[d] W8-16 = 30°C from 8-16 h after light onset; 20°C at all other times

[e] W16-24 = 30°C from 16-24 h after light onset; 20°C at all other times

[f] Mean initial body weight (g) ± standard error of the mean

TABLE IV

Initial body weight of green anole lizards in December prior to placement on experimental temperature and photoperiod regimes. "Hours after light onset" indicates the time of day that groups (individual cages) would be sacrificed 21 days later, and not time of day of initial weighing.

Regime	Hours after light onset 0	4	8	12	16	20	Means
WW[a]	5.12[f] ±.46	5.07 ±.42	5.14 ±.36	5.35 ±.47	5.18 ±.51	5.06 ±.40	5.15 ±.17
CC[b]	5.33 ±.36	5.34 ±.57	5.29 ±.35	5.20 ±.40	5.18 ±.41	5.27 ±.40	5.27 ±.16
W0-8[c]	5.13 ±.38	4.83 ±.46	4.57 ±.37	4.89 ±.33	4.99 ±.40	4.93 ±.34	4.89 ±.15
W8-16[d]	5.34 ±.43	4.90 ±.33	5.50 ±.46	4.97 ±.40	5.29 ±.41	5.34 ±.42	5.22 ±.16
W16-24[e]	5.04 ±.41	5.07 ±.52	5.18 ±.44	5.31 ±.39	4.83 ±.32	4.86 ±.29	5.02 ±.16

[a]WW = constant 30°C

[b]CC = constant 20°C

[c]W0-8 = 30°C from 0-8 h after light onset; 20°C at all other times

[d]W8-16 = 30°C from 8-16 h after light onset; 20°C at all other times

[e]W16-24 = 30°C from 16-24 h after light onset; 20°C at all other times

[f]Mean intial body weight (g) ± standard error of the mean

TABLE V

Effect of temperature regime on lizard final body weight in April. Green anoles were held on LD13:11 and on one of five temperature regimes. Groups of animals were sampled once every 4 h throughout a 24 h period.

Regime	Hours after light onset 0	4	8	12	16	20	Means[g]
WW[a]	4.30[f] ±.27	3.81 ±.23	4.66 ±.48	5.03 ±.27	4.65 ±.26	4.74 ±.29	4.53 ±.13
CC[b]	5.14 ±.33	5.55 ±.41	5.27 ±.49	5.13 ±.35	4.72 ±.19	5.26 ±.22	5.18 ±.14
W0-8[c]	5.10 ±.32	4.99 ±.43	4.48 ±.24	5.28 ±.39	4.82 ±.23	5.14 ±.31	4.97 ±.13
W8-16[d]	4.80 ±.25	5.64 ±.30	5.04 ±.28	4.78 ±.39	4.78 ±.22	4.78 ±.20	4.97 ±.12
W16-24[e]	5.13 ±.33	5.22 ±.38	5.72 ±.43	5.37 ±.36	5.14 ±.23	5.34 ±.54	5.32 ±.15

[a] WW = constant 30°C

[b] CC = constant 20°C

[c] W0-8 = 30°C from 0-8 h after light onset; 20°C at all other times

[d] W8-16 = 30°C from 8-16 h after light onset; 20°C at all other times

[e] W16-24 = 30°C from 16-24 h after light onset; 20°C at all other times

[f] Mean final body weight (g) ± standard error of the mean

[g] W16-24 CC W0-8 W8-16 WW ($P < .05$, Duncan's mrt)

TABLE VI

Effect of temperature regime on lizard final body weight in August. Green anoles were held on LD 13.5:10.5 and on one of five temperature regimes. Groups of animals were sampled once every 4 h throughout a 24 h period.

Regime	Hours after light onset 0	4	8	12	16	20	Means[g]
WW[a]	4.67[f] ±.39	4.95 ±.26	5.06 ±.28	4.84 ±.26	4.60 ±.23	4.48 ±.32	4.77 ±.13
CC[b]	5.05 ±.26	5.32 ±.46	5.48 ±.33	5.60 ±.37	5.15 ±.37	5.55 ±.30	5.37 ±.14
W0-8[c]	4.60 ±.29	4.26 ±.21	5.64 ±.51	5.38 ±.26	5.47 ±.21	4.59 ±.30	4.97 ±.14
W8-16[d]	4.76 ±.36	5.11 ±.37	4.87 ±.63	5.67 ±.23	4.90 ±.36	6.00 ±.44	5.20 ±.16
W16-24[e]	5.41 ±.31	4.95 ±.23	5.24 ±.34	4.93 ±.40	4.63 ±.22	4.96 ±.37	5.02 ±.13

[a]WW = constant 30°C

[b]CC = constant 20°C

[c]W0-8 = 30°C from 0-8 h after light onset; 20°C at all other times

[d]W8-16 = 30°C from 8-16 h after light onset; 20°C at all other times

[e]W16-24 = 30°C from 16-24 h after light onset; 20°C at all other times

[f]Mean final body weight (g) ± standard error of the mean

[g] CC W8-16 W16-24 W0-8 WW ($P < .05$, Duncan's mrt)

TABLE VII

Effect of temperature regime on lizard final body weights in December. Green anoles were held on LD 10.5:13.5 and on one of five temperature regimes. Groups of animals were sampled once every 4 h throughout a 24 h period.

Regime	Hours after light onset 0	4	8	12	16	20	Means
WW[a]	5.34[f] ±.48	5.53 ±.60	5.25 ±.20	5.52 ±.40	5.38 ±.37	6.06 ±.25	5.52 ±.16
CC[b]	5.68 ±.30	5.77 ±.54	5.72 ±.30	5.67 ±.30	5.66 ±.37	5.53 ±.34	5.67 ±.14
W0-8[c]	5.37 ±.38	4.95 ±.36	5.40 ±.42	5.15 ±.30	5.37 ±.34	5.14 ±.38	5.23 ±.14
W8-16[d]	5.57 ±.35	5.40 ±.40	6.00 ±.46	5.56 ±.42	5.74 ±.50	5.54 ±.28	5.68 ±.16
W16-24[e]	5.78 ±.35	5.84 ±.59	5.70 ±.45	5.92 ±.33	5.46 ±.36	5.53 ±.37	5.71 ±.16

[a] WW = constant 30°C

[b] CC = constant 20°C

[c] W0-8 = 30°C from 0-8 h after light onset; 20°C at all other times

[d] W8-16 = 30°C from 8-16 h after light onset; 20°C at all other times

[e] W16-24 = 30°C from 16-24 h after light onset; 20°C at all other times

[f] Mean body weight (g) ± standard error of the mean

TABLE VIII

Effect of temperature regime on lizard change in body weight (final body weight - initial body weight) in April. Animals were held on LD 13:11 and on one of five temperature regimes. Groups of animals were sampled once every 4 h throughout a 24 h period.

Regime	Hours after light onset 0	4	8	12	16	20	Means[g]
WW[a]	0.23[f] ±.20	-0.24 ±.18	0.44 ±.24	0.38 ±.28	0.25 ±.17	0.18 ±.18	0.21 ±.09
CC[b]	0.69 ±.11	0.68 ±.12	0.79 ±.19	0.50 ±.10	0.59 ±.16	0.96 ±.21	0.70 ±.06
W0-8[c]	0.72 ±.09	0.70 ±.33	0.38 ±.13	0.87 ±.09	0.53 ±.17	0.73 ±.17	0.66 ±.07
W8-16[d]	0.72 ±.12	1.15 ±.24	0.75 ±.15	0.54 ±.21	0.30 ±.18	0.50 ±.16	0.65 ±.08
W16-24[e]	0.55 ±.26	0.92 ±.27	1.23 ±.17	0.88 ±.39	0.91 ±.24	0.87 ±.43	0.90 ±.12

[a] WW = constant 30°C

[b] CC = constant 20°C

[c] W0-8 = 30°C from 0-8 h after light onset; 20°C at all other times

[d] W8-16 = 30°C from 8-16 h after light onset; 20°C at all other times

[e] W16-24 = 30°C from 16-24 h after light onset; 20°C at all other times

[f] Mean change in body weight (g) ± standard error of the mean

[g] W16-24 CC W0-8 W8-16 WW ($P < .05$, Duncan's mrt)

TABLE IX

Effect of temperature regime on lizard change in body weight in August. Green anoles were held on LD 13.5:10.5 and on one of five temperature regimes. Groups of animals were sampled once every 4 h throughout a 24 h period.

Regime	Hours after light onset 0	4	8	12	16	20	Means[g]
WW[a]	0.05[f] ±.15	0.51 ±.17	0.40 ±.31	0.12 ±.11	-0.26 ±.26	-0.24 ±.37	0.11 ±.10
CC[b]	0.06 ±.24	0.44 ±.14	0.35 ±.15	1.06 ±.21	0.56 ±.37	0.72 ±.12	0.55 ±.10
W0-8[c]	-0.16 ±.13	-0.51 ±.13	0.50 ±.29	1.00 ±.18	0.87 ±.11	-0.16 ±.21	0.25 ±.12
W8-16[d]	0.03 ±.30	0.19 ±.30	0.31 ±.35	0.87 ±.24	0.42 ±.17	0.84 ±.29	0.43 ±.12
W16-24[e]	0.75 ±.16	0.33 ±.12	0.60 ±.20	0.17 ±.27	-0.09 ±.12	-0.34 ±.43	0.24 ±.11

[a]WW = constant 30°C

[b]CC = constant 20°C

[c]W0-8 = 30°C from 0-8 h after light onset; 20°C at all other times

[d]W8-16 = 30°C from 8-16h after light onset; 20°C at all other times

[e]W16-24 = 30°C from 16-24 h after light onset; 20°C at all other times

[f]Mean change in body weight (g) ± standard error of the mean

[g] CC W8-16 W0-8 W16-24 WW ($P < .05$, Duncan's mrt)

TABLE X

Effect of temperature regime on lizard change in body weight in December. Green anoles were held on LD 10.5:13.5 and on one of five temperature regimes. Groups of animals were sampled once every 4 h throughout a 24 h period.

Regime	Hours after light onset 0	4	8	12	16	20	Means[g]
WW[a]	0.22[f] ±.19	0.46 ±.22	0.11 ±.30	0.17 ±.12	0.19 ±.18	1.00 ±.32	0.37 ±.10
CC[b]	0.36 ±.16	0.43 ±.09	0.43 ±.17	0.47 ±.18	0.48 ±.17	0.25 ±.12	0.40 ±.06
W0-8[c]	0.24 ±.17	0.12 ±.17	0.83 ±.24	0.26 ±.17	0.38 ±.16	0.21 ±.11	0.34 ±.08
W8-16[d]	0.23 ±.21	0.50 ±.17	0.50 ±.18	0.59 ±.14	0.45 ±.17	0.20 ±.26	0.41 ±.08
W16-24[e]	0.74 ±.26	0.77 ±.30	0.51 ±.34	0.61 ±.10	0.63 ±.13	0.67 ±.19	0.65 ±.09

[a] WW = constant 30°C

[b] CC = constant 20°C

[c] W0-8 = 30°C from 0-8 h after light onset; 20°C at all other times

[d] W8-16 = 30°C from 8-16 h after light onset; 20°C at all other times

[e] W16-24 = 30°C from 16-24 h after light onset; 20°C at all other times

[f] Mean change in body weight (g) ± standard error of the mean

[g] W16-24 W8-16 CC WW W0-8 ($P < .05$, Duncan's mrt)

TABLE XI

Effect of temperature regime on lizard testes wet weight in April. Green anoles were held on LD 13:11 and on one of five temperature regimes. Groups of animals were sampled once every 4 h throughout a 24 h period.

Regime	Hours after light onset 0	4	8	12	16	20	Means[g]
WW[a]	54±3[f]	53±4	50±5	53±2	53±6	72±10	56±3
CC[b]	77±4	90±3	83±9	82±5	75±4	73±2	80±2
W0-8[c]	64±5	66±4	72±5	80±7	65±3	77±5	71±2
W8-16[d]	72±5	79±5	76±7	69±3	83±4	68±4	74±2
W16-24[e]	77±2	76±5	79±7	81±7	76±5	75±3	78±2

[a]WW = constant 30°C

[b]CC = constant 20°C

[c]W0-8 = 30°C from 0-8 h after light onset; 20°C at all other times

[d]W8-16 = 30°C from 8-16 h after light onset; 20°C at all other times

[e]W16-24 = 30°C from 16-24 h after light onset; 20°C at all other times

[f]Mean testes wet weight (mg) ± standard error of the mean

[g] CC W16-24 W8-16 W0-8 WW ($P < .05$, Duncan's mrt)

TABLE XII

Effect of temperature regime on lizard testes wet weight in August. Green anoles were held on LD 13.5:10.5 and on one of five temperature regimes. Groups of animals were sampled once every 4 h throughout a 24 h period.

Regime	Hours after light onset 0	4	8	12	16	20	Means[g]
WW[a]	19±5[f]	20±6	10±2	16±7	16±5	20±2	17±2
CC[b]	42±5	33±7	42±5	36±6	32±7	36±4	36±2
W0-8[c]	14±3	14±4	24±9	25±4	15±5	12±2	17±2
W8-16[d]	18±4	15±4	34±9	32±6	19±5	20±3	23±2
W16-24[e]	12±4	26±5	15±3	26±6	12±2	29±5	19±2

[a]WW = constant 30°C

[b]CC = constant 20°C

[c]W0-8 = 30°C from 0-8 h after light onset; 20°C at all other times

[d]W8-16 = 30°C from 8-16 h after light onset; 20°C at all other times

[e]W16-24 = 30°C from 16-24 h after light onset; 20°C at all other times

[f]Mean testes wet weights (mg) ± standard error of the mean

[g] CC W8-16 W16-24 W0-8 WW ($P < .05$, Duncan's mrt)

TABLE XIII

Effect of temperature regime on lizard testes wet weights in December. Green anoles were held on LD 10.5:13.5 and on one of five temperature regimes. Groups of animals were sampled once every 4 h throughout a 24 h period.

Regime	Hours after light onset 0	4	8	12	16	20	Means[g]
WW[a]	41±8[f]	50±7	36±4	49±6	51±3	55±5	47±2
CC[b]	41±2	47±6	50±3	49±2	48±3	41±3	46±1
W0-8[c]	38±4	45±5	50±3	43±4	53±3	42±2	45±2
W8-16[d]	43±4	48±4	46±4	60±4	49±5	47±3	49±2
W16-24[e]	51±2	49±3	48±5	51±1	49±2	55±3	51±1

[a]WW = constant 30°C

[b]CC = constant 20°C

[c]W0-8 = 30°C from 0-8 h after light onset; 20°C at all other times

[d]W8-16 = 30°C from 8-16 h after light onset; 20°C at all other times

[e]W16-24 = 30°C from 16-24 h after light onset; 20°C at all other times

[f]Mean testes wet weights (mg) ± standard error of the mean

[g] W16-24 W8-16 WW CC W0-8 ($P < .05$, Duncan's mrt)

TABLE XIV

Effect of temperature regime on lizard gonadosomatic index (GSI) in April. Green anoles were held on LD 13:11 and on one of five regimes. Groups of animals were sampled once every 4 h throughout a 24 h period.

Regime	Hours after light onset 0	4	8	12	16	20	Means[g]
WW[a]	1.27[f] ±.07	1.40 ±.08	1.08 ±.06	1.05 ±.05	1.12 ±.11	1.51 ±.19	1.24 ±.05
CC[b]	1.54 ±.14	1.64 ±.08	1.56 ±.05	1.61 ±.10	1.59 ±.10	1.39 ±.06	1.54 ±.04
W0-8[c]	1.31 ±.17	1.36 ±.13	1.64 ±.09	1.57 ±.19	1.37 ±.09	1.50 ±.06	1.46 ±.05
W8-16[d]	1.51 ±.08	1.42 ±.10	1.50 ±.13	1.46 ±.06	1.72 ±.05	1.44 ±.10	1.51 ±.04
W16-24[e]	1.54 ±.11	1.49 ±.11	1.41 ± 16	1.51 ±.06	1.50 ±.08	1.47 ±.12	1.49 ±.04

[a] WW = constant 30°C

[b] CC = constant 20°C

[c] W0-8 = 30°C from 0-8 h after light onset; 20°C at all other times

[d] W8-16 = 30°C from 8-16 h after light onset; 20°C at all other times

[e] W16-24 = 30°C from 16-24 h after light onset; 20°C at all other times

[f] Mean GSI (testes weight / final body weight x 100) ± standard error of the mean

[g] CC W8-16 W16-24 W0-8 WW ($P < .05$, Duncan's mrt)

TABLE XV

Effect of temperature regime on lizard gonadosomatic index (GSI) in August. Green anoles were held on LD 13.5:10.5 and on one of five temperature regimes. Groups of animals were sampled once every 4 h throughout a 24 h period.

Regime	Hours after light onset 0	4	8	12	16	20	Means[g]
WW[a]	0.43[f] ±.10	0.40 ±.12	0.21 ±.06	0.34 ±.15	0.34 ±.09	0.46 ±.06	0.36 ±.04
CC[b]	0.82 ±.10	0.65 ±.13	0.76 ±.08	0.66 ±.12	0.61 ±.15	0.65 ±.07	0.69 ±.05
W0-8[c]	0.31 ±.04	0.31 ±.10	0.40 ±.11	0.46 ±.07	0.27 ±.09	0.25 ±.04	0.33 ±.03
W8-16[d]	0.40 ±.08	0.29 ±.08	0.67 ±.16	0.54 ±.08	0.38 ±.10	0.36 ±.07	0.43 ±.04
W16-24[e]	0.22 ±.07	0.53 ±.10	0.23 ±.04	0.57 ±.15	0.23 ±.07	0.58 ±.10	0.39 ±.04

[a] WW = constant 30°C

[b] CC = constant 20°C

[c] W0-8 = 30°C from 0-8 h after light onset; 20°C at all other times

[d] W8-16 = 30°C from 8-16 h after light onset; 20°C at all other times

[e] W16-24 = 30°C from 16-24 h after light onset; 20°C at all other times

[f] Mean GSI (testes weight / final body weight x 100) ± standard error of the mean

[g] CC W8-16 W16-24 WW W0-8 ($P < .05$, Duncan's mrt)

TABLE XVI

Effects of temperature regime on lizard gonadosomatic index (GSI) in December. Green anoles were held on LD 10.5:13.5 and on one of five temperature regimes. Groups of animals were sampled once every 4 h throughout a 24 h period.

Regime	Hours after light onset 0	4	8	12	16	20	Means
WW[a]	0.74[f] ±.14	0.88 ±.05	0.69 ±.08	0.89 ±.08	0.96 ±.05	0.91 ±.09	0.85 ±.04
CC[b]	0.72 ±.05	0.82 ±.10	0.88 ±.05	0.89 ±.07	0.86 ±.06	0.75 ±.05	0.82 ±.03
W0-8[c]	0.71 ±.06	0.92 ±.11	0.95 ±.08	0.84 ±.06	1.00 ±.03	0.86 ±.08	0.88 ±.03
W8-16[d]	0.78 ±.05	0.90 ±.06	0.76 ±.02	1.10 ±.11	0.88 ±.08	0.85 ±.06	0.88 ±.03
W16-24[e]	0.90 ±.05	0.87 ±.05	0.86 ±.09	0.87 ±.03	0.92 ±.06	1.00 ±.06	0.90 ±.02

[a] WW = constant 30°C

[b] CC = constant 20°C

[c] W0-8 = 30°C 0-8 h after light onset; 20°C at all other times

[d] W8-16 = 30°C 8-16 h after light onset; 20°C at all other times

[e] W16-24 = 30°C 16-24 h after light onset; 20°C at all other times

[f] Mean GSI (testes weight / final body weight x 100) ± standard error of the mean

TABLE XVII

Effect of temperature regime on the number of germ cell rows in lizard seminiferous tubules in April. Green anoles were held on LD 13:11 and on one of five temperature regimes. Groups of animals were sampled once every 4 h throughout a 24 h period.

Regime	Hours after light onset 0	4	8	12	16	20	Means[g]
WW[a]	5.7±.2[f]	5.8±.2	6.3±.2	5.8±.2	5.8±.5	5.3±.3	5.8±.1
CC[b]	8.2±.6	8.8±.3	8.7±.5	8.8±.3	8.5±.3	8.3±.2	8.6±.1
W0-8[c]	6.8±.2	8.0±.6	6.5±.2	7.8±.3	7.0±.4	7.8±.2	7.3±.2
W8-16[d]	6.8±.3	8.2±.5	8.4±.5	8.0±.2	7.5±.4	7.8±.4	7.8±.2
W16-24[e]	7.7±.2	8.5±.3	8.2±.3	8.2±.5	7.5±.5	7.8±.4	8.0±.2

[a] WW = constant 30°C

[b] CC = constant 20°C

[c] W0-8 = 30°C from 0-8 h after light onset; 20°C at all other times

[d] W8-16 = 30°C from 8-16 h after light onset; 20°C at all other times

[e] W16-24 = 30°C from 16-24 h after light onset; 20°C at all other times

[f] Mean number of germ cell rows ± standard error of the mean

[g] CC W16-24 W8-16 W0-8 WW ($P < .05$, Duncan's mrt)

TABLE XVIII

Effect of temperature regime on the number of germ cell rows in lizard seminiferous tubules in August. Green anoles were held on LD 13.5:10.5 and on one of five temperature regimes. Groups of animals were sampled once every 4 h throughout a 24 h period.

Regime	Hours after light onset 0	4	8	12	16	20	Means[g]
WW[a]	4.0[f] ±.4	3.0 ±.8	2.5 ±1.0	3.0 ±1.0	3.8 ±.6	3.6 ±.6	3.3 ±.3
CC[b]	6.8 ±.4	4.8 ±.5	5.3 ±.3	5.7 ±.5	4.8 ±.7	5.3 ±.7	5.4 ±.2
W0-8[c]	2.7 ±.5	2.8 ±.7	5.3 ±1.3	3.8 ±.9	3.4 ±1.1	2.0 ±.6	3.2 ±.4
W8-16[d]	3.8 ±.6	3.8 ±.9	4.6 ±1.0	5.6 ±.8	5.0 ±1.3	4.0 ±.7	4.5 ±.3
W16-24[e]	2.7 ±.7	5.0 ±.9	2.4 ±.7	4.6 ±.4	2.8 ±.8	6.0 ±.4	3.9 ±.3

[a] WW = constant 30°C

[b] CC = constant 20°C

[c] W0-8 = 30°C from 0-8 h after light onset; 20°C at all other times

[d] W8-16 = 30°C from 8-16 h after light onset; 20°C at all other times

[e] W16-24 = 30°C from 16-24 h after light onset; 20°C at all other times

[f] Mean number of germ cell rows ± standard error of the mean

[g] CC W8-16 W16-24 WW W0-8 ($P < .05$, Duncan's mrt)

TABLE XIX

Effect of temperature regime on the number of germ cell rows in lizard seminiferous tubules in December. Green anoles were held on LD 10.5:13.5 and on one of five temperature regimes. Groups of animals were sampled once every 4 h throughout a 24 h period.

Regime	Hours after light onset 0	4	8	12	16	20	Means[g]
WW[a]	9.0[f] ±2.0	8.7 ±.7	9.5 ±.4	9.2 ±.9	11.7 ±.9	10.0 ±1.0	9.7 ±.4
CC[b]	10.0 ±.9	10.8 ±.7	9.4 ±.5	10.3 ±.9	9.4 ±.5	9.2 ±.2	9.9 ±.3
W0-8[c]	10.0 ±.5	10.7 ±.5	10.2 ±.5	10.0 ±.3	11.4 ±.4	11.7 ±.3	10.6 ±.2
W8-16[d]	10.4 ±.8	9.4 ±.7	12.1 ±.9	10.4 ±.4	10.7 ±.8	10.4 ±.6	10.6 ±.3
W16-24[e]	10.6 ±.4	10.2 ±.4	10.8 ±.7	10.3 ±.9	10.4 ±.7	10.7 ±.3	10.5 ±.2

[a]WW = constant 30°C

[b]CC = constant 20°C

[c]W0-8 = 30°C from 0-8 h after light onset; 20°C at all other times

[d]W8-16 = 30°C from 8-16 h after light onset; 20°C at all other times

[e]W16-24 = 30°C from 16-24 h after light onset; 20°C at all other times

[f]Mean number of germ cell rows ± standard error of the mean

[g] W0-8 W8-16 W16-24 CC WW ($P < .05$, Duncan's mrt)

TABLE XX

Effect of temperature regime on lizard seminiferous tubule diameter in April. Green anoles were held on LD 13:11 and on one of five temperature regimes. Groups of animals were sampled once every 4 h throughout a 24 h period.

Regime	Hours after light onset 0	4	8	12	16	20	Means[g]
WW[a]	201±10[f]	194±8	205±6	189±7	219±11	194±8	200±4
CC[b]	232±8	243±3	243±5	228±11	230±12	246±10	237±3
W0-8[c]	208±5	227±5	227±7	220±7	244±7	233±7	228±3
W8-16[d]	221±10	207±13	201±9	213±10	195±4	220±5	209±4
W16-24[e]	225±9	240±13	242±9	241±6	224±8	245±5	236±3

[a]WW = constant 30°C

[b]CC = constant 20°C

[c]W0-8 = 30°C from 0-8 h after light onset; 20°C at all other times

[d]W8-16 = 30°C from 8-16 h after light onset; 20°C at all other times

[e]W16-24 = 30°C from 16-24 h after light onset; 20°C at all other times

[f]Mean seminiferous tubule diameter (μ) ± standard error of the mean

[g] CC W16-24 W0-8 W8-16 WW ($P < .05$, Duncan's mrt)

TABLE XXI

Effect of temperature regime on lizard seminiferous tubule diameter in August. Green anoles were held on LD 13.5:10.5 and on one of five temperature regimes. Groups of animals were sampled once every 4 h throughout a 24 h period.

Regime	Hours after light onset 0	4	8	12	16	20	Means[g]
WW[a]	156±16[f]	130±25	143±8	124±19	143±6	140±10	139±6
CC[b]	167±5	158±10	176±9	157±9	155±8	166±7	163±3
W0-8[c]	140±7	136±14	147±11	156±11	129±18	122±11	139±5
W8-16[d]	147±9	143±14	152±17	156±6	150±7	151±5	150±4
W16-24[e]	138±15	165±13	120±15	168±11	114±10	164±9	144±6

[a]WW = constant 30°C

[b]CC = constant 20°C

[c]W0-8 = 30°C from 0-8 h after light onset; 20°C at all other times

[d]W8-16 = 30°C from 8-16 h after light onset; 20°C at all other times

[e]W16-24 = 30°C from 16-24 h after light onset; 20°C at all other times

[f]Mean seminiferous tubule diameter (μ) ± standard error of the mean

[g] CC W8-16 W16-24 W0-8 WW ($P < .05$, Duncan's mrt)

TABLE XXII

Effects of temperature regime on lizard seminiferous tubule diameter in December. Green anoles were held on LD 10.5:13.5 and on one of five temperature regimes. Groups of animals were sampled once every 4 h throughout a 24 h period.

Regime	Hours after light onset 0	4	8	12	16	20	Means[g]
WW[a]	170±10[f]	174±5	146±2	154±9	165±10	173±7	163±3
CC[b]	166±2	181±5	163±6	164±17	165±6	153±4	167±3
W0-8[c]	182±5	174±3	150±7	153±3	159±5	165±3	165±3
W8-16[d]	162±9	160±6	170±6	174±6	181±9	183±4	172±3
W16-24[e]	158±7	169±2	162±9	174±3	180±7	176±5	171±3

[a]WW = constant 30°C

[b]CC = constant 20°C

[c]W0-8 = 30°C from 0-8 h after light onset; 20°C at all other times

[d]W8-16 = 30°C from 8-16 h after light onset; 20°C at all other times

[e]W16-24 = 30°C from 16-24 h after light onset; 20°C at all other times

[f]Mean seminiferous tubule diameter (μ) ± standard error of the mean

[g] W8-16 W16-24 CC W0-8 WW ($P < .05$, Duncan's mrt)

TABLE XXIII

Effect of temperature regime on lizard reproductive stage (see Table I) in April. Green anoles were held on LD 13:11 and on one of five temperature regimes. Groups of animals were sampled once every 4 h throughout a 24 h period.

Regime	Hours after light onset 0	4	8	12	16	20	Means[g]
WW[a]	6.80[f] ±.04	6.81 ±.06	6.80 ±.05	6.82 ±.02	6.85 ±.07	6.87 ±.06	6.83 ±.02
CC[b]	6.64 ±.07	6.61 ±.06	6.32 ±.07	6.40 ±.07	6.59 ±.08	6.72 ±.04	6.54 ±.03
W0-8[c]	6.66 ±.07	6.64 ±.04	6.80 ±.06	6.74 ±.08	6.72 ±.07	6.75 ±.03	6.72 ±.02
W8-16[d]	6.65 ±.09	6.58 ±.06	6.46 ±.09	6.51 ±.07	6.57 ±.10	6.65 ±.06	6.57 ±.03
W16-24[e]	6.72 ±.03	6.72 ±.07	6.67 ±.04	6.74 ±.05	6.63 ±.08	6.69 ±.08	6.69 ±.02

[a] WW = constant 30°C

[b] CC = constant 20°C

[c] W0-8 = 30°C from 0-8 h after light onset; 20°C at all other times

[d] W8-16 = 30°C from 8-16 h after light onset; 20°C at all other times

[e] W16-24 = 30°C from 16-24 h after light onset; 20°C at all other times

[f] Mean reproductive stage (see Table I) ± standard error of the mean

[g] WW W0-8 W16-24 W8-16 CC ($P < .05$, Duncan's mrt)

TABLE XXIV

Effect of temperature regime on lizard reproductive stage (see Table I) in August. Green anoles were held on LD 13.5:10.5 and on one of five temperature regimes. Groups of animals were sampled once every 4 h throughout a 24 h period.

Regime	Hours after light onset 0	4	8	12	16	20	Means[g]
WW[a]	7.31[f] ±.12	7.27 ±.22	7.47 ±.09	7.52 ±.21	7.42 ±.13	7.17 ±.16	7.36 ±.06
CC[b]	6.77 ±.13	6.77 ±.13	6.98 ±.08	6.89 ±.12	7.05 ±.14	6.98 ±.08	6.94 ±.04
W0-8[c]	7.35 ±.16	7.30 ±.18	7.09 ±.20	7.29 ±.12	7.27 ±.22	7.50 ±.10	7.30 ±.07
W8-16[d]	7.32 ±.15	7.29 ±.15	7.04 ±.25	7.11 ±.13	6.97 ±.22	7.12 ±.16	7.15 ±.07
W16-24[e]	7.43 ±.19	7.00 ±.13	7.58 ±.16	6.95 ±.14	7.46 ±.18	6.87 ±.10	7.23 ±.08

[a] WW = constant 30°C

[b] CC = constant 20°C

[c] W0-8 = 30°C from 0-8 h after light onset; 20°C at all other times

[d] W8-16 = 30°C from 8-16 h after light onset; 20°C at all other times

[e] W16-24 = 30°C from 16-24 h after light onset; 20°C at all other times

[f] Mean reproductive stage (see Table I) ± standard error of the mean

[g] WW W0-8 W16-24 W8-16 CC ($P < .05$, Duncan's mrt)

TABLE XXV

Effect of temperature regime on lizard reproductive stage (see Table I) in December. Green anoles were held on LD 10.5:13.5 and on one of five temperature regimes. Groups of animals were sampled once every 4 h throughout a 24 h period.

Regime	Hours after light onset 0	4	8	12	16	20	Means[g]
WW[a]	5.33[f] ±.33	5.93 ±.12	5.08 ±.23	5.08 ±.15	5.57 ±.17	5.06 ±.06	5.39 ±.10
CC[b]	4.65 ±.06	4.25 ±.08	4.39 ±.14	4.31 ±.19	4.28 ±.08	4.37 ±.16	4.37 ±.05
W0-8[c]	5.07 ±.25	4.89 ±.21	4.75 ±.12	4.60 ±.24	5.00 ±.13	4.50 ±.02	4.85 ±.08
W8-16[d]	5.03 ±.17	4.67 ±.08	4.89 ±.15	4.86 ±.12	5.06 ±.16	5.07 ±.21	4.93 ±.06
W16-24[e]	4.83 ±.12	4.40 ±.06	4.72 ±.20	4.55 ±.05	4.78 ±.10	4.68 ±.10	4.67 ±.05

[a] WW = constant 30°C

[b] CC = constant 20°C

[c] W0-8 = 30°C from 0-8 h after light onset; 20°C at all other times

[d] W8-16 = 30°C from 8-16 h after light onset; 20°C at all other times

[e] W16-24 = 30°C from 16-24 h after light onset; 20°C at all other times

[f] Mean reproductive stages (see Table I) ± standard error of the mean

[g] WW W8-16 W0-8 W16-24 CC ($P < .05$, Duncan's mrt)

TABLE XXVI

Effect of temperature regime on a lizard reproductive index (number of germ cell rows x seminiferous tubule diameter (μ)) in April. Green anoles were held on LD 13:11 and on one of five temperature regimes. Groups of animals were sampled once every 4 h throughout a 24 h period.

Regime	Hours after light onset 0	4	8	12	16	20	Means[g]
WW[a]	1144[f] ± 79	1138 ± 70	1351 ± 74	1100 ± 69	1300 ±155	1035 ± 80	1176 ± 39
CC[b]	1919 ±193	2145 ± 90	2112 ±147	2029 ±155	1956 ±126	2148 ± 74	2038 ± 52
W0-8[c]	1414 ± 35	1827 ±173	1505 ± 74	1723 ± 98	1717 ±122	1822 ± 66	1675 ± 48
W8-16[d]	1512 ±108	1683 ±144	1686 ±121	1715 ±123	1459 ± 74	1713 ± 83	1624 ± 46
W16-24[e]	1723 ± 81	2030 ±112	1982 ±115	1967 ±106	1692 ±170	1921 ±122	1886 ± 51

[a] WW = constant 30°C

[b] CC = constant 20°C

[c] W0-8 = 30°C from 0-8 h after light onset; 20°C at all other times

[d] W8-16 = 30°C from 8-16 h after light onset; 20°C at all other times

[e] W16-24 = 30°C from 16-24 h after light onset; 20°C at all other times

[f] Mean number of germ cell rows in the seminiferous tubules x seminiferous tubule diameter (μ) ± standard error of the mean

[g] CC W16-24 W0-8 W8-16 WW ($P < .05$, Duncan's mrt)

TABLE XXVII

Effect of temperature regime on a lizard reproductive index (number of germ cell rows x diameter of seminiferous tubules) in August. Green anoles were held on LD 13.5:10.5 and on one of five temperature regimes. Groups of animals were sampled once every 4 h throughout a 24 h period.

Regime	Hours after light onset 0	4	8	12	16	20	Means[g]
WW[a]	619[f] ± 81	465 ±172	381 ±124	426 ±226	557 ±102	501 ± 80	489 ± 55
CC[b]	1135 ± 73	782 ±125	939 ± 98	911 ±114	785 ±130	902 ±150	901 ± 49
W0-8[c]	375 ± 88	414 ±140	811 ±267	635 ±185	509 ±237	254 ± 85	487 ± 71
W8-16[d]	587 ±113	579 ±173	762 ±205	901 ±159	778 ±226	608 ±110	712 ± 67
W16-24[d]	425 ±135	873 ±176	328 ±139	788 ±118	340 ±106	1002 ±118	616 ± 68

[a] WW = constant 30°C

[b] CC = constant 20°C

[c] W0-8 = 30°C from 0-8 h after light onset; 20°C at all other times

[d] W8-16 = 30°C from 8-16 h after light onset; 20°C at all other times

[e] W16-24 = 30°C from 16-24 h after light onset; 20°C at all other times

[f] Mean number of germ cell rows in the seminiferous tubules x diameter of seminiferous tubules (μ) ± standard error of the mean

[g] CC W8-16 W16-24 WW W0-8 ($P < .05$, Duncan's mrt)

TABLE XXVIII

Effect of temperature regime on a lizard reproductive index (number of germ cell rows x diameter of seminiferous tubules) in December. Green anoles were held on LD 10.5:13.5 and on one of five temperature regimes. Groups of animals were sampled once every 4 h throughout a 24 h period.

Regime	Hours after light onset 0	4	8	12	16	20	Means[g]
WW[a]	1517[f] ±144	1524 ±142	1387 ± 71	1428 ±171	1948 ±316	1747 ±233	1574 ± 80
CC[b]	1655 ±132	1974 ±155	1547 ±109	1786 ±553	1550 ± 83	1411 ± 34	1656 ± 63
W0-8[c]	1912 ± 48	1878 ±122	1526 ± 93	1530 ± 62	1798 ±129	1923 ± 32	1753 ± 50
W8-16[d]	1704 ±179	1504 ±140	2083 ±190	1823 ±124	1964 ±237	1901 ± 99	1837 ± 68
W16-24[e]	1680 ± 99	1779 ± 49	1757 ±367	1803 ±186	1893 ±186	1884 ± 77	1811 ± 55

[a] WW = constant 30°C

[b] CC = constant 20°C

[c] W0-8 = 30°C from 0-8 h after light onset; 20°C at all other times

[d] W8-16 = 30°C from 8-16 h after light onset; 20°C at all other times

[e] W16-24 = 30°C from 16-24 h after light onset; 20°C at all other times

[f] Mean number of germ cell rows in the seminiferous tubules x diameter of seminiferous tubules (μ) ± standard error of the mean

[g] W8-16 W16-24 W0-8 CC WW ($P < .05$, Duncan's mrt)

TABLE XXIX

Effect of temperature regime on DNA content (µg) of lizard testis in April. Green anoles were held on LD 13:11 and on one of five temperature regimes. Groups of animals were sampled once every 4 h throughout a 24 h period.

Regime	Hours after light onset 0	4	8	12	16	20	Means[g]
WW[a]	414±38[f]	352±52	405±24	381±26	316±84	331±42	366±19
CC[b]	497±46	547±34	482±80	521±78	428±78	392±70	477±27
W0-8[c]	432±94	394±67	458±32	491±59	451±76	426±24	442±24
W8-16[d]	463±81	456±64	489±88	428±72	507±50	491±55	471±26
W16-24[e]	373±70	402±69	381±90	439±75	630±60	425±95	436±33

[a]WW = constant 30°C

[b]CC = constant 20°C

[c]W0-8 = 30°C from 0-8 h after light onset; 20°C at all other times

[d]W8-16 = 30°C from 8-16 h after light onset; 20°C at all other times

[e]W16-24 = 30°C from 16-24 h after light onset; 20°C at all other times

[f]Mean DNA (µg) per testis ± standard error of the mean

[g] CC W8-16 W0-8 W16-24 WW ($P < .05$, Duncan's mrt)

TABLE XXX

Effect of temperature regime on DNA content (µg) of lizard testis in August. Green anoles were held on LD 13.5:10.5 and on one of five temperature regimes. Groups of animals were sampled once every 4 h throughout a 24 h period.

Regime	Hours after light onset 0	4	8	12	16	20	Means[g]
WW[a]	120±41[f]	114±52	62±13	39±12	118±29	107±31	92±14
CC[b]	316±30	172±60	269±37	195±37	220±49	176±30	221±18
W0-8[c]	80±17	99±38	145±51	141±30	128±37	55±18	107±13
W8-16[d]	104±15	68±15	206±51	180±37	127±39	96±23	128±14
W16-24[e]	98±34	188±48	69±19	167±41	78±23	177±43	127±16

[a]WW = constant 30°C

[b]CC = constant 20°C

[c]W0-8 = 30°C from 0-8 h after light onset; 20°C at all other times

[d]W8-16 = 30°C from 8-16 h after light onset; 20°C at all other times

[e]W16-24 = 30°C from 16-24 h after light onset; 20°C at all other times

[f]Mean DNA (µg) per testis ± standard error of the mean

[g] CC W8-16 W16-24 W0-8 WW ($P < .05$, Duncan's mrt)

TABLE XXXI

Effect of temperature regime on DNA content (µg) of lizard testis in December. Green anoles were held on LD 10.5:13.5 and on one of five temperature regimes. Groups of animals were sampled once every 4 h throughout a 24 h period.

Regime	Hours after light onset 0	4	8	12	16	20	Means
WW[a]	253±50[f]	281±40	172±22	258±46	282±48	269±48	252±17
CC[b]	177±39	298±50	286±35	273±14	222±30	208±29	244±15
W0-8[c]	173±29	237±26	162±57	238±38	249±42	237±43	216±16
W8-16[d]	232±28	129±38	238±26	256±42	267±40	261±47	231±16
W16-24[e]	319±30	216±46	267±50	225±48	270±28	280±37	263±16

[a]WW = constant 30°C

[b]CC = constant 20°C

[c]W0-8 = 30°C from 0-8 h after light onset; 20°C at all other times

[d]W8-16 = 30°C from 8-16 h after light onset; 20°C at all other times

[e]W16-24 = 30°C from 16-24 h after light onset; 20°C at all other times

[f]Mean DNA (µg) per testis ± standard error of the mean

TABLE XXXII

Effect of temperature regime on ^{3}H-thymidine incorporation into DNA of lizard testes in April. Green anoles were held on LD 13:11 and on one of five temperature regimes. Groups of animals were sampled once every 4 h throughout a 24 h period.

Regime	Hours after light onset 0	4	8	12	16	20	Means
WW[a]	567[f] ±112	660 ±57	884 ±86	621 ±97	569 ±134	632 ±127	656 ±44
CC[b]	542 ±73	1197 ±562	543 ±55	544 ±123	1591 ±758	451 ±91	819 ±169
W0-8[c]	861 ±202	475 ±117	691 ±59	782 ±128	781 ±150	711 ±87	713 ±52
W8-16[d]	838 ±193	658 ±93	491 ±189	606 ±102	754 ±92	903 ±66	708 ±55
W16-24[e]	425 ±158	682 ±165	379 ±112	682 ±164	909 ±158	583 ±259	601 ±73

[a]WW = constant 30°C

[b]CC = constant 20°C

[c]W0-8 = 30°C from 0-8 h after light onset; 20°C at all other times

[d]W8-16 = 30°C from 8-16 h after light onset; 20°C at all other times

[e]W16-24 = 30°C from 16-24 h after light onset; 20°C at all other times

[f]Mean counts per minute (^{3}H-thymidine incorporation into DNA of 0.14 of one testis) ± standard error of the mean

TABLE XXXIII

Effect of temperature regime on ^{3}H-thymidine incorporation into DNA of lizard testes in August. Green anoles were held on LD 13.5:10.5 and on one of five temperature regimes. Groups of animals were sampled once every 4 h throughout a 24 h period.

Regime	Hours after light onset 0	4	8	12	16	20	Means
WW[a]	562±159[f]	334±200	233±142	74±16	168±67	212±91	272±57
CC[b]	236±68	114±12	201±58	172±72	220±64	170±52	183±23
W0-8[c]	62±9	65±20	114±15	75±16	88±10	62±16	77±6
W8-16[d]	74±18	66±8	283±54	155±11	111±21	81±27	123±16
W16-24[e]	100±26	193±89	158±41	129±50	104±23	238±93	154±25

[a]WW = constant 30°C

[b]CC = constant 20°C

[c]W0-8 = 30°C from 0-8 h after light onset; 20°C at all other times

[d]W8-16 = 30°C from 8-16 h after light onset; 20°C at all other times

[e]W16-24 = 30°C from 16-24 h after light onset; 20°C at all other times

[f]Mean counts per minute (^{3}H-thymidine incorporation into DNA of 0.14 of one testis) ± standard error of the mean

[g] WW CC W16-24 W8-16 W0-8 ($P < .05$, Duncan's mrt)

TABLE XXXIV

Effect of temperature regime on ^{3}H-thymidine incorporation into DNA of lizard testes in December. Green anoles were held on LD 10.5:13.5 and on one of five temperature regimes. Groups of animals were sampled once every 4 h throughout a 24 h period.

Regime	Hours after light onset 0	4	8	12	16	20	Means[g]
WW[a]	931[f] ±232	641 ±106	604 ± 80	1026 ±263	833 ±203	468 ±165	739 ±75
CC[b]	880 ±242	1146 ±279	544 ±176	1223 ±290	939 ±175	1005 ±117	956 ±91
W0-8[c]	763 ±177	868 ±172	293 ±65	837 ±158	730 ±157	891 ±201	730 ±69
W8-16[d]	1023 ±170	338 ±99	939 ±244	640 ±153	990 ±143	885 ±155	807 ±79
W16-24[e]	1337 ±296	679 ±106	763 ±197	622 ±163	832 ±180	770 ±183	834 ++83

[a]WW = constant 30°C

[b]CC = constant 20°C

[c]W0-8 = 30°C from 0-8 h after light onset; 20°C at all other times

[d]W8-16 = 30°C from 8-16 h after light onset; 20°C at all other times

[e]W16-24 = 30°C from 16-24 h after light onset; 20°C at all other times

[f]Mean counts per minute (^{3}H-thymidine incorporation into DNA of 0.14 of one testis) ±standard error of the mean

[g] CC W16-24 W8-16 W0-8 WW ($P < .05$, Duncan's mrt)

TABLE XXXV

Effect of temperature regime on lizard testis DNA content (mg) per g testis wet weight in April. Green anoles were held on LD 13:11 and on one of five temperature regimes. Groups of animals were sampled once every 4 h throughout a 24 h period.

Regime	Hours after light onset 0	4	8	12	16	20	Means
WW[a]	15.3[f] ±1.1	12.9 ±1.2	16.9 ±1.8	14.7 ±1.3	11.1 ±2.3	10.1 ±1.7	13.5 ±0.7
CC[b]	13.0 ±0.7	12.3 ±1.2	11.8 ±1.6	12.7 ±1.9	12.1 ±2.8	10.8 ±1.9	12.1 ±0.7
W0-8[c]	12.3 ±3.6	12.2 ±2.0	12.5 ±0.7	12.6 ±1.6	13.8 ±2.3	11.2 ±0.7	12.8 ±0.8
W8-16[d]	12.7 ±2.1	11.4 ±1.2	12.8 ±1.9	12.7 ±2.1	12.5 ±2.1	13.6 ±1.2	12.6 ±0.7
W16-24[e]	9.9 ±2.0	11.0 ±1.9	9.6 ±2.3	10.8 ±1.7	16.5 ±1.7	11.6 ±2.7	11.4 ±0.9

[a]WW = constant 30°C

[b]CC = constant 20°C

[c]W0-8 = 30°C from 0-8 h after light onset; 20°C at all other times

[d]W8-16 = 30°C from 8-16 h after light onset; 20°C at all other times

[e]W16-24 = 30°C from 16-24 h after light onset; 20°C at all other times

[f]Mean DNA content (mg) of testis / testis wet weights (g) ± standard error of the mean

TABLE XXXVI

Effect of temperature regime on lizard testis DNA content (mg) per g testis wet weight in August. Green anoles were held on LD 13.5:10.5 and on one of five temperature regimes. Groups of animals were sampled once every 4 h throughout a 24 h period.

Regime	Hours after light onset 0	4	8	12	16	20	Means
WW[a]	13.3[f] ±2.4	12.4 ±3.0	12.8 ±1.4	8.9 ±3.9	15.8 ±1.6	10.1 ±2.4	12.2 ±1.0
CC[b]	15.6 ±1.0	11.7 ±2.7	12.7 ±0.9	10.9 ±1.3	18.7 ±4.4	10.3 ±2.1	13.3 ±1.1
W0-8[c]	10.7 ±0.9	14.3 ±3.0	12.6 ±2.1	10.9 ±0.9	24.2 ±7.5	8.6 ±1.2	13.5 ±1.6
W8-16[d]	12.0 ±1.3	10.7 ±2.2	12.1 ±2.8	11.5 ±1.2	14.8 ±2.6	9.2 ±1.9	11.7 ±0.8
W16-24[e]	18.9 ±3.8	11.9 ±5.0	13.1 ±5.0	13.4 ±1.6	12.1 ±1.3	11.7 ±1.4	13.6 ±1.2

[a]WW = constant 30°C

[b]CC = constant 20°C

[c]W0-8 = 30°C from 0-8 h after light onset; 20°C at all other times

[d]W8-16 = 30°C from 8-16 h after light onset; 20°C at all other times

[e]W16-24 = 30°C from 16-24 h after light onset; 20°C at all other times

[f]Mean DNA content (mg) of testis / testis wet weight (g) ± standard error of the mean

TABLE XXXVII

Effect of temperature regime on lizard testis DNA content (mg) per g testis wet weight in December. Green anoles were held on LD 10.5:13.5 and on one of five temperature regimes. Groups of animals were sampled once every 4 h throughout a 24 h period.

Regime	Hours after light onset 0	4	8	12	16	20	Means
WW[a]	13.7[f] ±1.6	12.5 ±1.9	10.0 ±1.4	11.3 ±2.3	11.7 ±2.2	10.3 ±1.9	11.5 ±0.7
CC[b]	8.9 ±2.0	14.7 ±3.5	11.8 ±1.5	11.1 ±0.6	9.4 ±1.6	10.3 ±1.4	11.0 ±0.8
W0-8[c]	9.7 ±1.8	10.8 ±0.8	6.3 ±2.2	11.5 ±1.8	9.1 ±1.2	11.6 ±2.3	9.8 ±0.7
W8-16[d]	10.9 ±1.4	5.8 ±1.9	10.6 ±1.2	8.6 ±1.4	10.9 ±1.4	11.1 ±1.7	9.6 ±0.6
W16-24[e]	12.6 ±1.4	8.8 ±1.7	11.8 ±2.1	10.0 ±2.0	11.0 ±1.2	10.3 ±1.3	10.6 ±0.7

[a] WW = constant 30°C

[b] CC = constant 20°C

[c] W0-8 = 30°C from 0-8 h after light onset; 20°C at all other times

[d] W8-16 = 30°C from 8-16 h after light onset; 20°C at all other times

[e] W16-24 = 30°C from 16-24 h after light onset; 20°C at all other times

[f] Mean DNA content (mg) of testis / testis wet weight (g) ± standard error of the mean

TABLE XXXVIII

Effect of temperature regime on rate of DNA replication in lizard testis / g DNA in April. Green anoles were held on LD 13:11 and on one of five temperature regimes. Groups of animals were sampled once every 4 h throughout a 24 h period.

Regime	Hours after light onset 0	4	8	12	16	20	Means
WW[a]	9.6[f] ±1.5	14.0 ±1.4	15.5 ±1.7	11.3 ±1.4	14.2 ±2.9	13.0 ±2.1	12.9 ±0.8
CC[b]	7.9 ±1.4	14.9 ±6.6	8.8 ±1.3	7.6 ±1.3	21.9 ±9.1	7.7 ±0.9	11.6 ±2.0
W0-8[c]	13.5 ±1.2	9.0 ±1.8	10.9 ±1.4	11.2 ±1.5	11.8 ±0.8	11.6 ±1.2	11.3 ±0.5
W8-16[d]	12.8 ±1.9	10.4 ±1.3	5.9 ±1.7	9.9 ±0.2	10.7 ±1.6	13.2 ±0.9	10.6 ±0.7
W16-24[e]	8.4 ±2.4	11.0 ±1.5	10.1 ±3.5	11.5 ±2.2	9.9 ±1.2	8.8 ±2.7	10.0 ±0.9

[a]WW = constant 30°C

[b]CC = constant 20°C

[c]W0-8 = 30°C from 0-8 h after light onset; 20°C at all other times

[d]W8-16 = 30°C from 8-16 h after light onset; 20°C at all other times

[e]W16-24 = 30°C from 16-24 h after light onset; 20°C at all other times

[f]Mean counts per minute (^{3}H-thymidine incorporation into DNA per testis) / g DNA in testis ± standard error of the mean

TABLE XXXIX

Effect of temperature regime on rate of DNA replication in the lizard testis / g DNA in August. Green anoles were held on LD 13.5:10.5 and on one of five temperature regimes. Groups of animals were sampled once every 4 h throughout a 24 h period.

Regime	Hours after light onset 0	4	8	12	16	20	Means[g]
WW[a]	27.2[f] ±8.6	21.8 ±3.9	15.0 ±5.9	19.4 ±8.0	13.1 ±6.9	16.0 ±4.9	18.4 ±2.6
CC[b]	5.2 ±1.6	7.3 ±1.5	5.5 ±1.3	6.0 ±1.6	6.4 ±0.8	7.1 ±1.4	6.3 ±0.5
W0-8[c]	7.7 ±2.5	5.9 ±1.4	8.1 ±2.1	4.2 ±0.6	7.6 ±2.4	9.6 ±1.8	7.2 ±0.8
W8-16[d]	5.4 ±1.4	9.2 ±2.0	7.7 ±1.5	6.2 ±0.6	9.0 ±3.2	6.2 ±1.7	7.3 ±0.8
W16-24[e]	11.5 ±3.9	8.2 ±3.4	20.4 ±4.3	5.6 ±1.3	12.5 ±3.8	10.9 ±3.6	11.7 ±1.6

[a] WW = constant 30°C

[b] CC = constant 20°C

[c] W0-8 = 30°C from 0-8 h after light onset; 20°C at all other times

[d] W8-16 = 30°C from 8-16 h after light onset; 20°C at all other times

[e] W16-24 = 30°C from 16-24 h after light onset; 20°C at all other times

[f] Mean counts per minute (^{3}H-thymidine incorporation into DNA per testis) / g DNA in testis ± standard error of the mean

[g] WW W16-24 W8-16 W0-8 CC ($P < .05$, Duncan's mrt)

TABLE XL

Effect of temperature regime on rate of DNA replication in the lizard testis / g DNA in December. Green anoles were held on LD 10.5:13.5 and on one of five temperature regimes. Groups of animals were sampled once every 4 h throughout a 24 h period.

Regime	Hours after light onset 0	4	8	12	16	20	Means[g]
WW[a]	32.1[f] ±9.8	15.6 ±1.0	25.2 ±3.6	26.2 ±3.5	19.3 ±2.4	14.6 ±6.4	21.8 ±2.2
CC[b]	32.9 ±3.8	26.6 ±4.6	13.0 ±3.0	31.3 ±6.9	31.5 ±6.3	36.5 ±5.8	28.7 ±2.3
W0-8[c]	29.5 ±2.7	26.5 ±4.7	15.4 ±2.3	23.5 ±2.9	22.0 ±3.6	25.5 ±2.9	23.8 ±1.4
W8-16[d]	29.7 ±2.8	18.8 ±2.2	25.7 ±6.6	16.6 ±1.4	26.2 ±1.9	25.3 ±4.0	23.9 ±1.6
W16-24[e]	28.1 ±4.9	24.1 ±2.4	22.1 ±4.9	18.7 ±2.1	21.8 ±4.4	18.7 ±3.6	22.3 ±1.6

[a]WW = constant 30°C

[b]CC = constant 20°C

[c]W0-8 = 30°C from 0-8 h after light onset; 20°C at all other times

[d]W8-16 = 30°C from 8-16 h after light onset; 20°C at all other times

[e]W16-24 = 30°C from 16-24 h after light onset; 20°C at all other times

[f]Mean counts per minute (^{3}H-thymidine incorporation into DNA per Testis) / g DNA in testis ± standard error of the mean

[g] CC W8-16 W0-8 W16-24 WW ($P < .05$, Duncan's mrt)

TABLE XLI

Effect of temperature regime on lizard fat body weight in April. Green anoles were held on LD 13:11 and on one of five temperature regimes. Groups of animals were sacrificed once every 4 h throughout a 24 h period.

Regime	Hours after light onset 0	4	8	12	16	20	Means[g]
WW[a]	51±12[f]	31±9	46±21	36±12	29±8	38±7	37±5
CC[b]	51±9	47±18	32±16	42±15	33±9	42±9	41±5
W0-8[c]	41±14	47±17	41±10	71±17	63±17	35±8	46±6
W8-16[d]	72±17	94±31	50±14	56±17	50±9	61±10	64±7
W16-24[e]	49±19	42±12	38±10	50±10	23±4	27±8	38±5

[a]WW = constant 30°C

[b]CC = constant 20°C

[c]W0-8 = 30°C from 0-8 h after light onset; 20°C at all other times

[d]W8-16 = 30°C from 8-16 h after light onset; 20°C at all other times

[e]W16-24 = 30°C from 16-24 h after light onset; 20°C at all other times

[f]Mean fat body wet weight (mg) ± standard error of the mean

[g] W8-16 W0-8 CC W16-24 WW ($P < .05$, Duncan's mrt)

TABLE XLII

Effect of temperature regime on lizard fat body weight in August. Green anoles were held on LD 13.5:10.5 and on one of five temperature regimes. Groups of animals were sacrificed once every 4 h throughout a 24 h period.

Regime	Hours after light onset 0	4	8	12	16	20	Means
WW[a]	96±30[f]	63±20	80±24	53±25	44±17	42±22	60±9
CC[b]	46±13	78±30	63±20	49±26	84±22	93±17	68±9
W0-8[c]	96±40	29±17	109±63	95±12	104±21	33±10	74±12
W8-16[d]	37±14	119±15	50±20	100±23	85±25	108±32	73±10
W16-24[e]	115±31	38±27	79±29	38±13	35±13	35±13	54±9

[a]WW = constant 30°C

[b]CC = constant 20°C

[c]W0-8 = 30°C from 0-8 h after light onset; 20°C at all other times

[d]W8-16 = 30°C from 8-16 h after light onset; 20°C at all other times

[e]W16-24 = 30°C from 16-24 h after light onset; 20°C at all other times

[f]Mean fat body wet weight (mg) ± standard error of the mean

TABLE XLIII

Effect of temperature regime on lizard fat body wet weight in December. Green anoles were held on LD 10.5:13.5 and on one of five temperature regimes. Groups of animals were sampled once every 4 h throughout a 24 h period.

Regime	Hours after light onset 0	4	8	12	16	20	Means[g]
WW[a]	108±27[f]	104±37	117±18	160±12	92±12	179±17	136±10
CC[b]	146±12	186±57	143±28	122±18	135±20	135±18	145±12
W0-8[c]	133±28	117±10	128±16	108±14	111±17	126±22	121±7
W8-16[d]	116±18	123±16	131±7	158±25	136±14	173±11	140±7
W16-24[e]	136±16	186±27	149±17	120±13	122±18	143±16	143±8

[a] WW = constant 30°C

[b] CC = constant 20°C

[c] W0-8 = 30°C from 0-8 h after light onset; 20°C at all other times

[d] W8-16 = 30°C from 8-16 h after light onset; 20°C at all other times

[e] W16-24 = 30°C from 16-24 h after light onset; 20°C at all other times

[f] Mean fat body wet weight (mg) ± standard error of the mean

[g] W16-24 CC W8-16 WW W0-8 ($P < .05$, Duncan's mrt)

TABLE XLIV

Effect of temperature regime on lizard fat body wet weight expressed as percent body weight in April. Green anoles were held on LD 13:11 and on one of five temperature regimes. Groups of animals were sampled once every 4h throughout a 24 h period.

Regime	Hours after light onset 0	4	8	12	16	20	Means[g]
WW[a]	1.14[f] ±.22	0.63 ±.20	o.82 ±.33	0.66 ±.20	0.61 ±.17	0.73 ±.12	0.77 ±.09
CC[b]	0.99 ±.16	0.78 ±.24	0.52 ±.21	0.77 ±.24	0.67 ±.17	0.80 ±.18	0.76 ±.08
W0-8[c]	0.82 ±.27	0.75 ±.28	0.72 ±.21	1.31 ±.25	1.28 ±.30	0.54 ±.14	0.90 ±.11
W8-16[d]	1.44 ±.29	1.60 ±.42	0.97 ±.26	1.08 ±.28	1.01 ±.17	1.28 ±.20	1.24 ±.11
W16-24[e]	0.89 ±.31	0.76 ±.17	0.67 ±.15	0.91 ±.17	0.45 ±.07	0.50 ±.12	0.70 ±.07

[a] WW = constant 30°C

[b] CC = constant 20°C

[c] W0-8 = 30°C from 0-8 h after light onset; 20°C at all other times

[d] W8-16 = 30°C from 8-16 h after light onset; 20°C at all other times

[e] W16-24 = 30°C from 16-24 h after light onset; 20°C at all other times

[f] Mean fat body weight expressed as percent body weight (fat body wet weight (g) / final body weight (g) x 100 ± standard error of mean

[g] W8-16 W0-8 WW CC W16-24 ($P < .05$, Duncan's mrt)

TABLE XLV

Effect of temperature regime on lizard fat body wet weight expressed as percent body weight in August. Green anoles were held on LD 13.5:10.5 and on one of five temperature regimes. Groups of animals were sampled once every 4 h throughout a 24 h period.

Regime	Hours after light onset 0	4	8	12	16	20	Means
WW[a]	1.59[f] ±.48	1.27 ±.37	1.55 ±.46	.83 ±.34	.74 ±.32	.82 ±.38	1.17 ±.17
CC[b]	.88 ±.23	1.10 ±.39	1.14 ±.35	.80 ±.42	1.54 ±.33	1.64 ±.23	1.19 ±.14
W0-8[c]	1.57 ±.68	.64 ±.39	1.69 ±.83	1.82 ±.27	1.84 ±.34	.71 ±.22	1.37 ±.20
W8-16[d]	.59 ±.23	1.54 ±.42	.90 ±.29	1.75 ±.41	1.66 ±.42	1.33 ±.48	1.30 ±.16
W16-24[e]	2.04 ±.53	.73 ±.18	1.36 ±.47	.61 ±.23	.56 ±.24	.56 ±.32	.98 ±.16

[a] WW = constant 30°C

[b] CC = constant 20°C

[c] W0-8 = 30°C from 0-8 h after light onset; 20°C at all other times

[d] W8-16 = 30°C from 8-16 h after light onset; 20°C at all other times

[e] W16-24 = 30°C from 16-24 h after light onset; 20°C at all other times

[f] Mean fat body weight expressed as percent body weight (fat body wet wet weight (g) / final body weight (g) x 100) ± standard error of the mean

TABLE XLVI

Effects of temperature regime on lizard fat body wet weight expressed as percent body weight in December. Green anoles were held on LD 10.5: 13.5 and on one of five temperature regimes. Groups of animals were sampled once every 4 h throughout a 24 h period.

Regime	Hours after light onset 0	4	8	12	16	20	Means
WW[a]	1.91[f] ±.44	2.10 ±.49	2.25 ±.35	2.95 ±.24	2.28 ±.21	2.96 ±.28	2.41 ±.15
CC[b]	2.56 ±.12	2.95 ±.59	2.42 ±.41	2.16 ±.31	2.40 ±.32	2.45 ±.30	2.49 ±.15
W0-8[c]	2.47 ±.41	2.47 ±.30	2.33 ±.17	2.08 ±.19	2.04 ±.31	2.35 ±.30	2.29 ±.11
W8-16[d]	2.05 ±.29	2.27 ±.27	2.28 ±.23	2.77 ±.33	2.42 ±.24	3.17 ±.25	2.49 ±.12
W16-24[e]	2.36 ±.26	3.11 ±.21	2.71 ±.33	2.03 ±.21	2.19 ±.25	2.58 ±.23	2.49 ±.11

[a]WW = constant 30°C

[b]CC = constant 20°C

[c]W0-8 = 30°C from 0-8 h after light onset; 20°C at all other times

[d]W8-16 = 30°C from 8-16 h after light onset; 20°C at all other times

[e]W16-24 = 30°C from 16-24 h after light onset; 20°C at all other times

[f]Mean fat body weight expressed as percent body weight (fat body wet weight (g) / final body weight (g) x 100) ± standard error of the mean

TABLE XLVII

Effect of temperature regime on lizard fat body lipid weight in April. Green anoles were held on LD 13:11 and on one of five temperature regimes. Groups of animals were sampled once every 4 h throughout a 24 h period.

Regime	Hours after light onset 0	4	8	12	16	20	Means[g]
WW[a]	37±9[f]	23±6	34±15	27±9	20±6	22±5	27±4
CC[b]	33±5	32±13	24±12	32±12	23±6	29±7	29±4
W0-8[c]	30±11	32±13	27±6	53±13	38±5	22±6	34±4
W8-16[d]	35±11	39±12	33±7	43±8	53±14	71±24	46±6
W16-24[e]	33±14	30±8	25±7	34±8	13±2	18±5	26±3

[a]WW = constant 30°C

[b]CC = constant 20°C

[c]W0-8 = 30°C from 0-8 h after light onset; 20°C at all other times

[d]W8-16 = 30°C from 8-16 h after light onset; 20°C at all other times

[e]W16-24 = 30°C from 16-24 h after light onset; 20°C at all other times

[f]Mean fat body lipid weight (mg) ± standard error of the mean

[g] W8-16 W0-8 CC WW W16-24 ($P < .05$, Duncan's mrt)

TABLE XLVIII

Effect of temperature regime on lizard fat body lipid weight in August. Green anoles were held on LD 13.5:10.5 and on one of five temperature regimes. Groups of animals were sampled once every 4 h throughout a 24 h period.

Regime	Hours after light onset 0	4	8	12	16	20	Means
WW[a]	77±23[f]	49±17	61±20	38±20	31±13	28±17	49±8
CC[b]	29±6	60±23	47±15	39±20	59±16	64±13	53±7
W0-8[c]	77±32	22±13	81±48	71±27	77±18	22±7	57±10
W8-16[d]	26±10	89±13	39±15	74±19	66±21	83±25	62±8
W16-24[e]	92±24	26±8	60±23	28±10	25±9	25±15	45±8

[a]WW = constant 30°C

[b]CC = constant 20°C

[c]W0-8 = 30°C from 0-8 h after light onset; 20°C at all other times

[d]W8-16 = 30°C from 8-16 h after light onset; 20°C at all other times

[e]W16-24 = 30°C from 16-24 h after light onset; 20°C at all other times

[f]Mean fat body lipid weight (mg) ± standard error of the mean

TABLE XLIX

Effect of temperature regime on lizard fat body lipid weight in December. Green anoles were held on LD 10.5:13.5 and on one of five temperature regimes. Groups of animals were sampled once every 4 h throughout a 24 h period.

Regime	Hours after light onset 0	4	8	12	16	20	Means
WW[a]	83±21[f]	99±32	90±14	117±9	92±12	130±15	101±8
CC[b]	111±8	141±47	108±21	90±15	103±15	103±13	109±9
W0-8[c]	104±21	82±8	98±13	80±10	85±14	96±17	91±6
W8-16[d]	88±14	92±12	98±6	110±19	102±11	128±9	105±5
W16-24[e]	100±11	138±20	113±15	87±10	94±15	108±13	107±6

[a]WW = constant 30°C

[b]CC = constant 20°C

[c]W0-8 = 30°C from 0-8 h after light onset; 20°C at all other times

[d]W8-16 = 30°C from 8-16 h after light onset; 20°C at all other times

[e]W16-24 = 30°C from 16-24 h after light onset; 20°C at all other times

[f]Mean fat body lipid weight (mg) ± standard error of the mean

TABLE L

Effect of temperature regime on percent lipid within lizard fat bodies in April. Green anoles were held on LD 13:11 and on one of five temperature regimes. Groups of animals were sampled once every 4 h throughout a 24 h period.

Regime	Hours after light onset 0	4	8	12	16	20	Means[g]
WW[a]	68±4[f]	75±2	74±1	77±4	69±2	65±3	71±1
CC[b]	66±2	64±3	74±4	77±2	70±3	74±1	70±1
W0-8[c]	73±6	67±4	69±4	73±2	75±4	71±8	71±2
W8-16[d]	71±3	74±2	67±3	71±2	64±5	69±2	70±1
W16-24[e]	65±4	68±4	68±1	66±5	56±6	66±6	66±2

[a]WW = constant 30°C

[b]CC = constant 20°C

[c]W0-8 = 30°C from 0-8 h after light onset; 20°C at all other times

[d]W8-16 = 30°C from 8-16 h after light onset; 20°C at all other times

[e]W16-24 = 30°C from 16-24 h after light onset; 20°C at all other times

[f]Mean percent lipid in fat bodies (lipid weight (mg) / fat body wet weight (mg) x 100) ± standard error of the mean

[g] WW W0-8 CC W8-16 W16-24 ($P < .05$, Duncan's mrt)

TABLE LI

Effect of temperature regime on percent lipid within lizard fat bodies in August. Green anoles were held on LD 13.5:10.5 and on one of five temperature regimes. Groups of animals were sampled once every 4 h throughout a 24 h period.

Regime	Hours after light onset 0	4	8	12	16	20	Means
WW[a]	81±2[f]	77±4	74±3	74±1	68±3	67±5	74±1
CC[b]	80±2	76±1	74±8	79±3	68±3	68±3	74±1
W0-8[c]	77±3	72±4	72±2	77±2	76±2	69±6	74±1
W8-16[d]	71±3	74±2	78±5	74±1	77±1	78±3	75±1
W16-24[e]	81±2	72±2	73±3	74±2	72±2	68±3	74±1

[a]WW = constant 30°C

[b]CC = constant 20°C

[c]W0-8 = 30°C from 0-8 h after light onset; 20°C at all other times

[d]W8-16 = 30°C from 8-16 h after light onset; 20°C at all other times

[e]W16-24 = 30°C from 16-24 h after light onset; 20°C at all other times

[f]Mean percent lipid in fat bodies (lipid weight (mg) / fat body wet weight (mg) x 100) ± standard error of the mean

TABLE LII

Effect of temperature regime on percent lipid within lizard fat bodies in December. Green anoles were held on LD 10.5:13.5 and on one of five temperature regimes. Groups of animals were sampled once every 4 h throughout a 24 h period.

Regime	Hours after light onset 0	4	8	12	16	20	Means
WW[a]	76±1[f]	77±3	77±1	73±1	75±2	75±1	75±1
CC[b]	76±1	74±3	75±1	73±2	76±2	77±1	75±1
W0-8[c]	78±2	70±1	77±1	74±1	77±2	76±1	75±1
W8-16[d]	75±2	74±2	75±1	70±1	75±1	74±2	74±1
W16-24[e]	74±1	74±1	75±2	74±1	76±1	74±2	74±1

[a]WW = constant 30°C

[b]CC = constant 20°C

[c]W0-8 = 30°C from 0-8 h after light onset; 20°C at all other times

[d]W8-16 = 30°C from 8-16 h after light onset; 20°C at all other times

[e]W16-24 = 30°C from 16-24 h after light onset; 20°C at all other times

[f]Mean percent lipid in fat bodies (lipid weight (mg) / fat body wet weight (mg) x 100) ± standard error of the mean

TABLE LIII

Effect of temperature regime on ^{3}H-acetate incorporation into lizard fat bodies in April. Green anoles were held on LD 13:11 and on one of five temperature regimes. Groups of animals were sampled once every 4 h throughout a 24 h period.

Regime	Hours after light onset 0	4	8	12	16	20	Means[g]
WW[a]	249[f] ± 77	249 ± 70	491 ±222	283 ± 94	237 ± 59	790 ±567	387 ±105
CC[b]	566 ±141	295 ± 72	1846 ±646	268 ± 74	238 ± 61	286 ± 44	584 ±146
W0-8[c]	788 ±282	667 ±362	380 ±107	322 ± 94	1405 ±864	666 ±273	698 ±174
W8-16[d]	3085 ±1328	523 ±110	1707 ±578	208 ± 57	343 ±101	393 ± 87	1024 ±288
W16-24[e]	791 ±511	896 ±365	277 ± 60	311 ± 73	1044 ±198	384 ±202	611 ±119

[a] WW = constant 30°C

[b] CC = constant 20°C

[c] W0-8 = 30°C from 0-8 h after light onset; 20°C at all other times

[d] W8-16 = 30°C from 8-16 h after light onset; 20°C at all other times

[e] W16-24 = 30°C from 16-24 h after light onset; 20°C at all other times

[f] Mean counts per minute (^{3}H-acetate incorporation into lipids of paired fat bodies) ± standard error of the mean

[g] W8-16 W0-8 W16-24 CC WW ($P < .05$, Duncan's mrt)

TABLE LIV

Effect of temperature regime on ^{3}H-acetate incorporation into lizard fat bodies in August. Green anoles were held on LD 13.5:10.5 and on one of five temperature regimes. Groups of animals were sampled once every 4 h throughout a 24 h period.

Regime	Hours after light onset 0	4	8	12	16	20	Means[g]
WW[a]	330[f] ± 74	154 ± 38	555 ±288	661 ±343	253 ± 61	130 ± 18	350 ± 81
CC[b]	283 ±151	252 ± 72	236 ± 33	895 ±580	618 ±327	1714 ±572	683 ±171
W0-8[c]	196 ± 51	114 ± 22	920 ±405	1970 ±568	1673 ±742	310 ± 50	882 ±207
W8-16[d]	1713 ±1215	389 ± 56	276 ± 85	290 ± 54	439 ± 87	871 ±544	659 ±233
W16-24[e]	412 ±167	292 ± 73	1087 ±377	264 ± 83	319 ±189	126 ± 45	437 ± 95

[a] WW = constant 30°C

[b] CC = constant 20°C

[c] W0-8 = 30°C from 0-8 h after light onset; 20°C at all other times

[d] W8-16 = 30°C from 8-16 h after light onset; 20°C at all other times

[e] W16-24 = 30°C from 16-24 h after light onset; 20°C at all other times

[f] Mean counts per minute (^{3}H-acetate incorporation into lipids of paired fat bodies) ± standard error of the mean

[g] W0-8 CC W8-16 W16-24 WW ($P < .05$, Duncan's mrt)

TABLE LV

Effect of temperature regime on ^{3}H-acetate incorporation into lizard fat bodies in December. Green anoles were held on LD 10.5:13.5 and on one of five temperature regimes. Groups of animals were sampled once every 4 h throughout a 24 h period.

Regime	Hours after light onset 0	4	8	12	16	20	Means
WW[a]	151±30[f]	218±36	205±62	108±17	150±61	397±138	209±29
CC[b]	231±76	532±301	411±216	127±9	550±383	200±27	342±88
W0-8[c]	170±23	830±633	151±29	206±25	428±189	171±32	326±110
W8-16[d]	206±25	178±21	383±165	145±23	124±13	181±27	203±30
W16-24[e]	207±44	207±57	501±227	178±34	393±205	667±293	359±73

[a]WW = constant 30°C

[b]CC = constant 20°C

[c]W0-8 = 30°C from 0-8 h after light onset; 20°C at all other times

[d]W8-16 = 30°C from 8-16 h after light onset; 20°C at all other times

[e]W16-24 = 30°C from 16-24 h after light onset; 20°C at all other times

[f]Mean counts per minute (^{3}H-acetate incorporation into lipids of paired fat bodies) ± standard error of the mean

TABLE LVI

Effect of temperature regime on ^{3}H-acetate incorporation into lizard fat bodies / g lipid in fat bodies in April. Green anoles were held on LD 13:11 and on one of five temperature regimes. Groups of animals were sampled once every 4 h throughout a 24 h period.

Regime	Hours after light onset 0	4	8	12	16	20	Means
WW[a]	9,582[f] ±3,872	12,756 ±2,886	39,845 ±26,907	32,351 ±20,773	16,700 ±3,738	39,677 ±26,756	21,078 ±5,830
CC[b]	16,623 ±3,728	43,145 ±33,604	97,421 ±25,256	14,815 ±5,241	26,325 ±14,815	17,081 ±6,961	36,453 ±8,817
W0-8[c]	44,609 ±20,344	23,598 ±6,698	14,359 ±3,512	6,514 ±1,106	39,440 ±26,513	38,203 ±15,111	23,305 ±6,567
W8-16[d]	72,407 ±28,802	9,788 ±2,669	84,442 ±43,443	7,345 ±2,135	12,651 ±3,706	8,814 ± 744	31,093 ±9,121
W16-24[e]	37,675 ±17,380	65,600 ±43,037	15,046 ±5,188	9,475 ±1,235	98,198 ±34,060	36,676 ±23,646	43,779 ±10,919

[a]WW = constant 30°C

[b]CC = constant 20°C

[c]W0-8 = 30°C from 0-8 h after light onset; 20°C at all other times

[d]W8-16 = 30°C from 8-16 h after light onset; 20°C at all other times

[e]W16-24 = 30°C from 16-24 h after light onset; 20°C at all other times

[f]Mean counts per minute (^{3}H-acetate incorporation for paired fat bodies) / lipid content (g) of paired fat bodies

TABLE LVII

Effects of temperature regime on ^{3}H-acetate incorporation into lizard fat bodies / g lipid in fat bodies in August. Green anoles were held on LD 13.5:10.5 and on one of five temperature regimes. Groups of animals were sampled once every 4 h throughout a 24 h period

Regime	Hours after light onset 0	4	8	12	16	20	Means
WW[a]	6,669[f] ±1,991	11,089 ±7,623	9,400 ±2,043	34,209 ±12,048	13,204 ±5,957	42,432 ±23,195	19,189 ±5,002
CC[b]	12,455 ±3,880	14,056 ±8,543	14,307 ±10,317	93,504 ±57,467	13,381 ±6,104	32,212 ±13,056	32,263 ±12,123
W0-8[c]	6,692 ±3,025	39,686 ±15,591	14,581 ±1,404	43,990 ±26,137	25,685 ±11,377	20,758 ±4,361	26,099 ±5,835
W8-16[d]	78,558 ±30,894	4,730 ± 893	11,707 ±3,453	6,078 ±2,158	13,527 ±6,182	8,993 ±3,023	20,316 ±7,176
W16-24[e]	11,503 ±6,194	23,340 ±8,237	32,915 ±9,177	14,654 ±3,903	18,402 ±8,030	7,668 ±1,117	18,171 ±2,988

[a] WW = constant 30°C

[b] CC = constant 20°C

[c] W0-8 = 30°C from 0-8 h after light onset; 20°C at all other times

[d] W8-16 = 30°C from 8-16 h after light onset; 20°C at all other times

[e] W16-24 = 30°C from 16-24 h after light onset; 20°C at all other times

[f] Mean counts per minute (^{3}H-acetate incorporation for paired fat bodies) / lipid content (g) of paired fat bodies

TABLE LVIII

Effect of temperature regime on ^{3}H-acetate incorporation into lizard fat bodies / g lipid in fat bodies in December. Green anoles were held on LD 10.5:13.5 and on one of five temperature regimes. Groups of animals were sampled once every 4 h throughout a 24 h period.

Regime	Hours after light onset 0	4	8	12	16	20	Means
WW[a]	3,938[f] ±2,047	6,026 ±3,196	2,386 ± 583	977 ±204	2,234 ± 812	3,159 ± 985	3,153 ± 700
CC[b]	2,414 ± 951	5,866 ±3,150	3,967 ±1,482	1,619 ±239	5,512 ±3,486	2,192 ± 378	3,595 ± 827
W0-8[c]	2,647 ±1,018	11,619 ±9,083	1,769 ± 415	2,653 ± 188	7,453 ±3,500	2,538 ±1,014	4,780 ±1,640
W8-16[d]	2,993 ± 759	2,394 ± 611	4,376 ±2,065	1,935 ± 667	1,292 ± 160	1,490 ± 268	2,413 ± 408
W16-24[e]	2,069 ± 341	1,894 ± 710	6,382 ±3,866	2,282 ± 600	4,718 ±2,022	8,115 ±4,176	4,243 ±1,025

[a] WW = constant 30°C

[b] CC = constant 20°C

[c] W0-8 = 30°C from 0-8 h after light onset; 20°C at all other times

[d] W8-16 = 30°C from 8-16 h after light onset; 20°C at all other times

[e] W16-24 = 30°C from 16-24 h after light onset; 20°C at all other times

[f] Mean counts per minute / g lipid in lizard fat bodies ± standard error of the mean

TABLE LIX

Summary of stimulatory temperature regimes for nine physiological variables measured in the male green anole at three different times of year. Only significant differences are given. Temperature regimes underlined were significantly higher ("high") or lower ("low") than all other regimes. Numbers under each column give significant differences among seasons (1=highest mean levels)

	April		August		December	
	high	low	high	low	high	low
final body weight		WW	CC W8-16	WW		
	2		2		1	
weight gain		WW	CC W8-16	WW W0-8 W16-24	W16-24	
	1		3		2	
gonad wet weight	CC W16-24	WW W0-8	CC		W16-24	W0-8
	1		3		2	
number of germ cell rows	CC W16-24	WW W0-8	CC W8-16	WW W0-8	W0-8 W8-16 W16-24	WW
	2		3		1	
reproductive stage	WW W0-8 W16-24	CC W8-16	WW	CC W8-16	WW W8-16	CC W16-24
	2		1		3	
DNA content of testis	CC W8-16	WW	CC			
	1		3		2	
rate of DNA replication			WW	W0-8	CC	WW
	2		3		1	
fat body weight	W8-16				W16-24	W0-8
	3		2		1	
rate of lipogenesis	W8-16	WW	W0-8	WW W16-24		
	1		1		2	

TABLE LX

Summary of the effects of five temperature regimes on lizard weight gain (g) at three different times of year. For explanation of temperature regimes, see Table II.

Regime	April[a]	August[b]	December[c]
WW	0.21±0.09[d]	0.11±0.10	0.37±0.10
CC	0.70±0.06	0.55±0.10	0.40±0.06
W0-8	0.66±0.07	0.25±0.12	0.34±0.08
W8-16	0.65±0.08	0.43±0.12	0.41±0.08
W16-24	0.90±0.12	0.24±0.11	0.65±0.09

[a] W16-24 CC W0-8 W8-16 WW ($P < .05$, Duncan's mrt)

[b] CC W8-16 W0-8 W16-24 WW ($P < .05$, Duncan's mrt)

[c] W16-24 W8-16 CC WW W0-8 ($P < .05$, Duncan's mrt)

[d] Mean weight gain (final body weight (g) - initial body weight (g)) ± standard error of the mean

TABLE LXI

Summary of the effects of five temperature regimes on lizard testes wet weights (mg) at three different times of year. For explanation of temperature regimes, see Table II.

Regime	April[a]	August[b]	December[c]
WW	56±3[d]	17±2	47±2
CC	80±2	36±2	46±1
W0-8	71±2	17±2	45±2
W8-16	74±2	23±2	49±2
W16-24	78±2	19±2	51±1

[a] CC W16-24 W8-16 W0-8 WW ($P < .05$, Duncan's mrt)

[b] CC W8-16 W16-24 W0-8 WW ($P < .05$, Duncan's mrt)

[c] W16-24 W8-16 WW CC W0-8 ($P < .05$, Duncan's mrt)

[d]Mean paired testes wet weights (mg) ± standard error of the mean

TABLE LXII

Summary of the effects of five temperature regimes on lizard reproductive stage at three different times of year. For explanation of temperature regimes and stages, see Tables I and II.

Regime	April[a]	August[b]	December[c]
WW	6.83±0.02[d]	7.36±0.06	5.39±0.10
CC	6.54±0.03	6.94±0.04	4.37±0.05
W0-8	6.72±0.02	7.30±0.07	4.85±0.08
W8-16	6.57±0.03	7.15±0.07	4.93±0.06
W16-24	6.69±0.02	7.23±0.08	4.67±0.05

[a] WW W0-8 W16-24 W8-16 CC ($P < .05$, Duncan's mrt)

[b] WW W0-8 W16-24 W8-16 CC ($P < .05$, Duncan's mrt)

[c] WW W8-16 W0-8 W16-24 CC ($P < .05$, Duncan's mrt)

[d] Mean reproductive stage (see Table I) ± standard error of the mean

TABLE LXIII

Summary of the effects of five temperature regimes on ^{3}H-thymidine incorporation into DNA of lizard testes at three different times of year. For explanation of temperature regimes, see Table II.

Regime	April	August[a]	December[b]
WW	656±44[c]	272±57	739±75
CC	819±169	183±23	956±91
W0-8	713±52	77±6	730±69
W8-16	708±55	123±16	807±79
W16-24	601±73	154±25	834±83

[a] WW CC W16-24 W8-16 W0-8 ($P < .05$, Duncan's mrt)

[b] CC W16-24 W8-16 W0-8 WW ($P < .05$, Duncan's mrt)

[c] Mean counts per minute (^{3}H-thymidine incorporation into DNA of 0.14 of one testis) ± standard error of the mean

TABLE LXIV

Summary of the effects of five temperature regimes on ^{3}H-acetate incorporation (as counts per minute) into lipid of lizard fat bodies at three different times of year. For explanation of temperature regimes, see Table II.

Regime	April[a]	August[b]	December
WW	387±105[c]	350±81	209±29
CC	584±146	683±171	342±88
W0-8	698±174	882±207	326±110
W8-16	1024±288	659±233	203±30
W16-24	611±119	437±95	359±73

[a] W8-16 W0-8 W16-24 CC WW ($P < .05$, Duncan's mrt)

[b] W0-8 CC W8-16 W16-24 WW ($P < .05$, Duncan's mrt)

[c] Mean counts per minute (^{3}H-acetate incorporation into lipid of paired fat bodies) ± standard error of the mean

Figure 1. Time of day differences in testicular DNA replication (^{3}H-thymidine incorporation into DNA of 0.14 of one testis). Green anoles were held on LD 13:11 and on one of five temperature regimes in April. Groups of animals were sampled and sacrificed once every 4 h for 24 h. Bottom horizontal bar represents the photoperiod; top three short bars represent the heat phase of the thermoperiod. Lines connect mean counts per minute (^{3}H-thymidine incorporation into DNA per testicular aliquot); vertical lines are one standard error of the mean. Temperature regimes are: WW = constant 30°C; CC = constant 20°C; WO-8 = 30°C from 0-8 h after light onset, 20°C at all other times; W8-16 = 30°C from 8-16 h after light onset, 20°C at all other times; W16-24 = 30°C from 16-24 h after light onset, 20°C at all other times.

Levels peaked at 16-20 h after light onset in the thermoperiod groups, and 16 h after light onset in the CC group ($P < .05$, Student's t test).

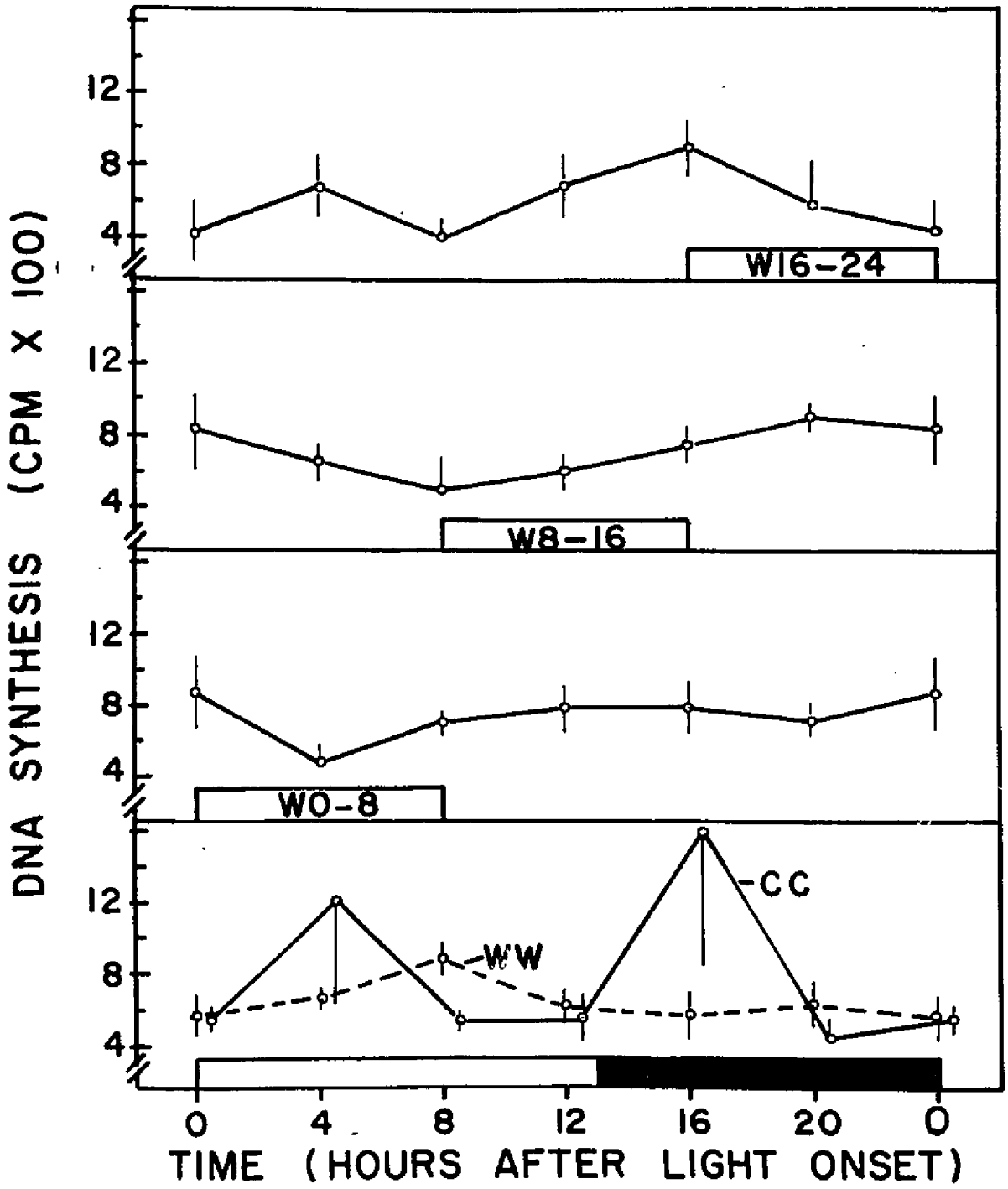

DNA SYNTHESIS (CPM X 100)
12
8
4
W16-24
W8-16
W0-8
CC
WW
0
4
8
12
16
20
0
TIME (HOURS AFTER LIGHT ONSET)

Figure 2. Time of day differences in testicular DNA replication as counts per minute (^{3}H-thymidine incorporation into DNA of 0.14 of one testis). Green anoles were held on LD 13.5:10.5 and on one of five temperature regimes in August (see Figure 1 for explanation). Groups of animals were sampled and sacrificed once every 4 h throughout a 24 h period.

Levels troughed 20 h after heat onset and at light onset in the thermoperiod groups, and peaked at light onset in the WW group ($P < .05$, Student's t test).

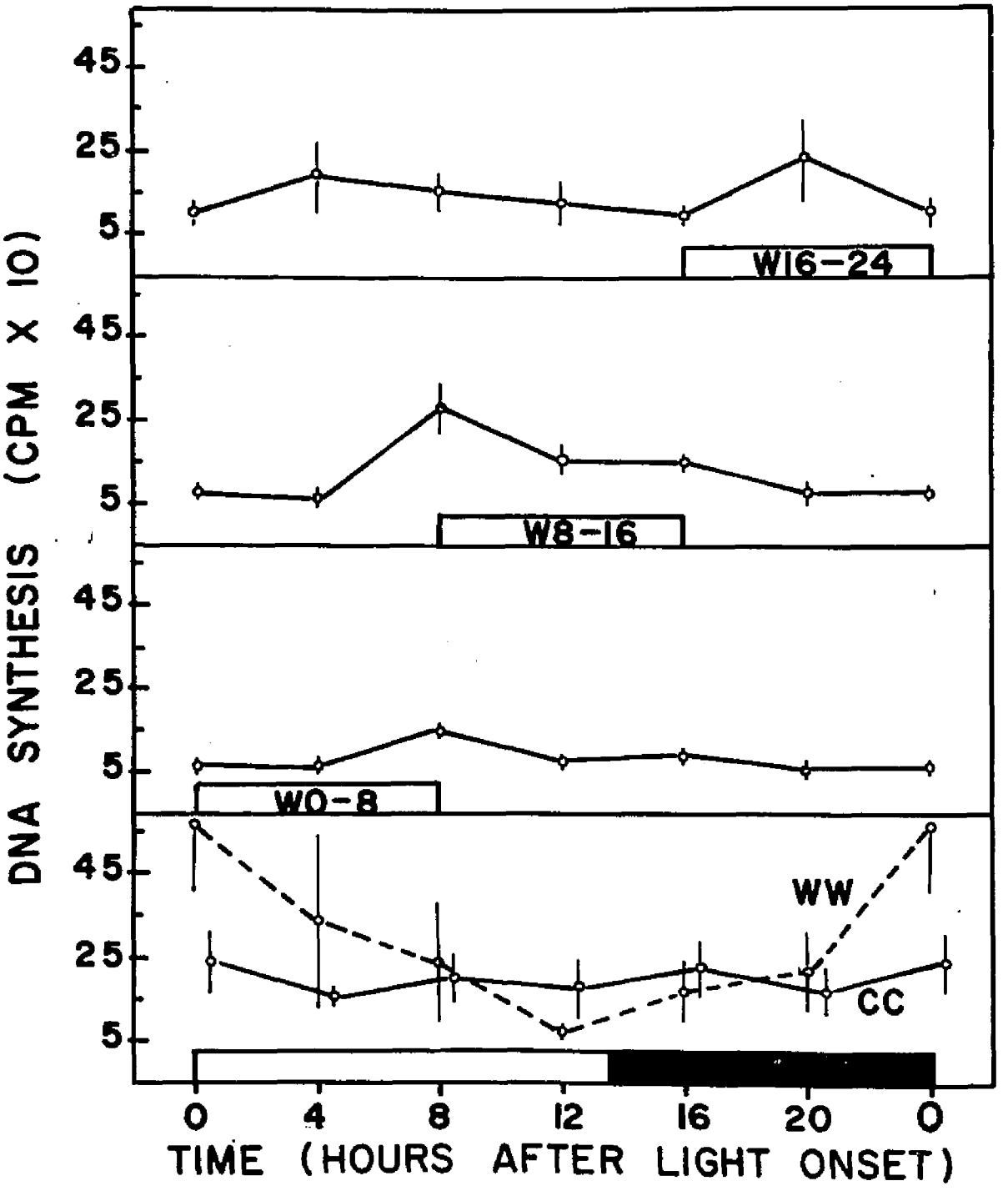

DNA SYNTHESIS (CPM X 10)
45
25
5
W16-24
W8-16
W0-8
WW
CC
0
4
8
12
16
20
0
TIME (HOURS AFTER LIGHT ONSET)

Figure 3. Time of day differences in testicular DNA replication as counts per minute (^{3}H-thymidine incorporation into DNA of 0.14 of one testis). Green anoles were held on LD 10.5:13.5 and on one of five temperature regimes in December (see Figure 1 for explanation). Groups of animals were sampled and sacrificed once every 4 h throughout a 24 h period.

Levels troughed at 8 h after light onset in CC and WO-8, and at 4 h after light onset in W8-16. Levels peaked at light onset in W16-24 and at 0 and 12 h after light onset in WW ($P < .05$, Student's t test).

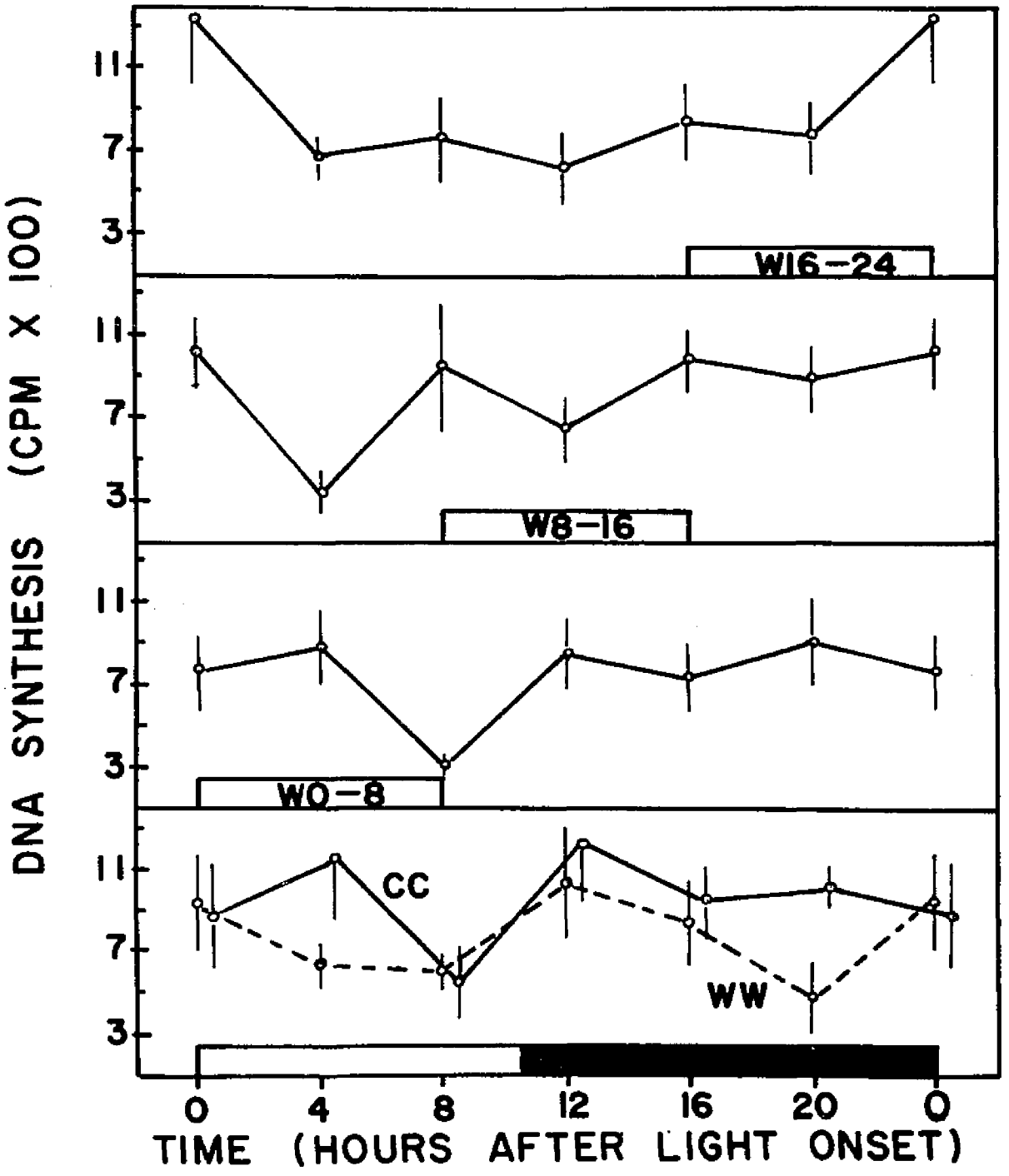

DNA SYNTHESIS (CPM X 100)
11
7
3
W16–24
W8–16
W0–8
CC
WW
0
4
8
12
16
20
0
TIME (HOURS AFTER LIGHT ONSET)

Figure 4. Time of day differences in fat body lipogenesis (^{3}H-acetate incorporation into lizard fat body lipid) in April. Green anoles were held on LD 13:11 and on one of five temperature regimes (see Figure 1 for explanation). Groups of animals were sampled and sacrificed once every 4 h for 24 h. Means are counts per minute ^{3}H- acetate incorporation into lipids of paired fat bodies.

Counts per minute peaked 8 h after light onset in CC and WW. Levels peaked 16 h after heat onset in W0-8 and W8-16, and at heat onset in W16-24; levels troughed 8 h after light onset in the thermoperiod groups ($P < .05$, Student's t test).

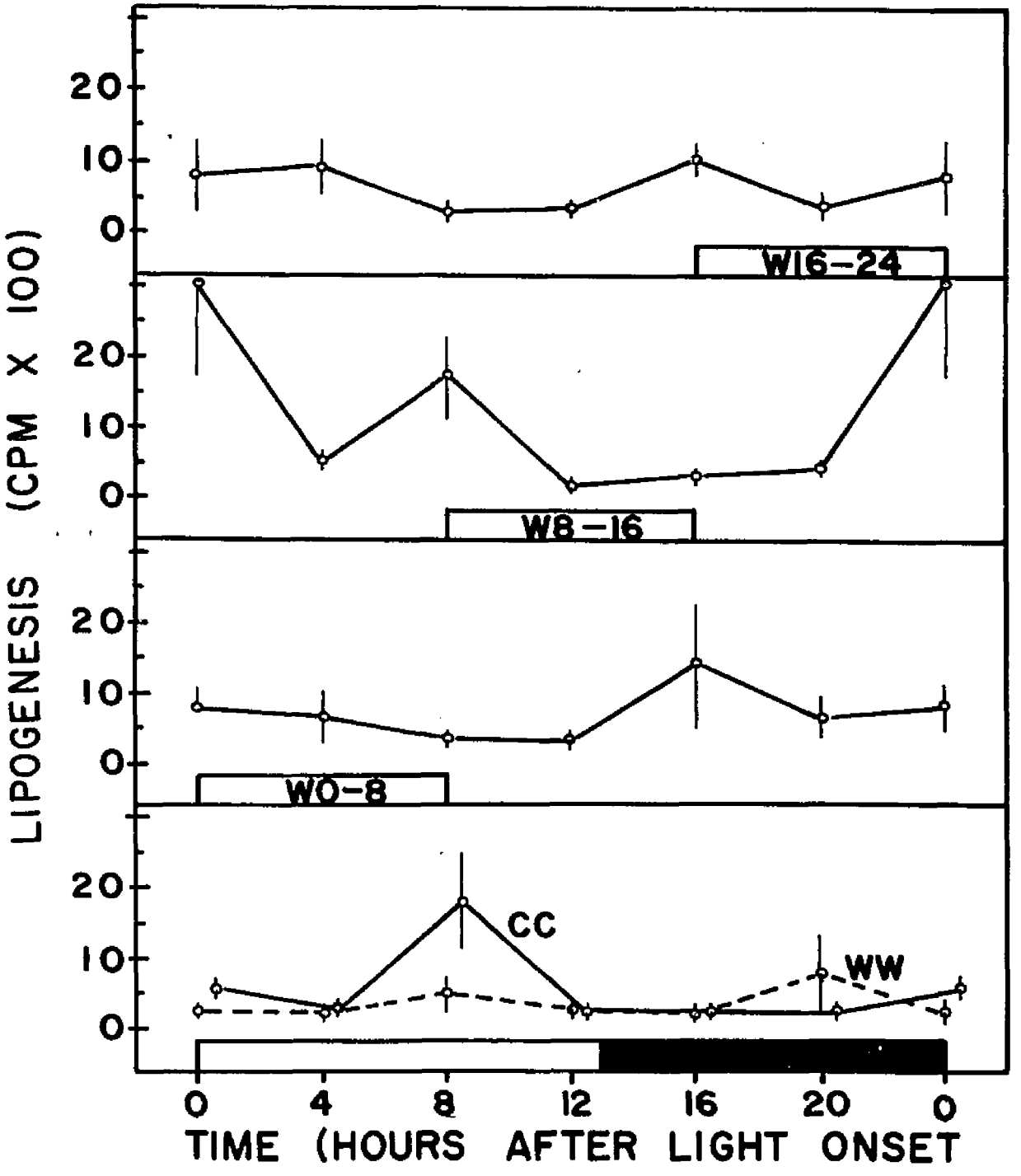

LIPOGENESIS (CPM X 100)
20
10
0
W16-24
W8-16
W0-8
CC
WW
0
4
8
12
16
20
0
TIME (HOURS AFTER LIGHT ONSET

Figure 5. Time of day differences in fat body lipogenesis (^{3}H-acetate incorporation into lizard fat body lipid)in August. Green anoles were held on LD 13.5:10.5 and on one of five temperature regimes (see Figure 1 for explanation). Groups of animals were sampled and sacrificed once every 4 h for 24 h. Means are counts per minute ^{3}H-acetate incorporation into lipids of paired fat bodies.

Counts per minute were highest 20 h after light onset in the CC group, and 12 h after light onset for WW. Levels peaked 16 h after heat onset in the thermoperiod groups ($P < .05$, Student's t test).

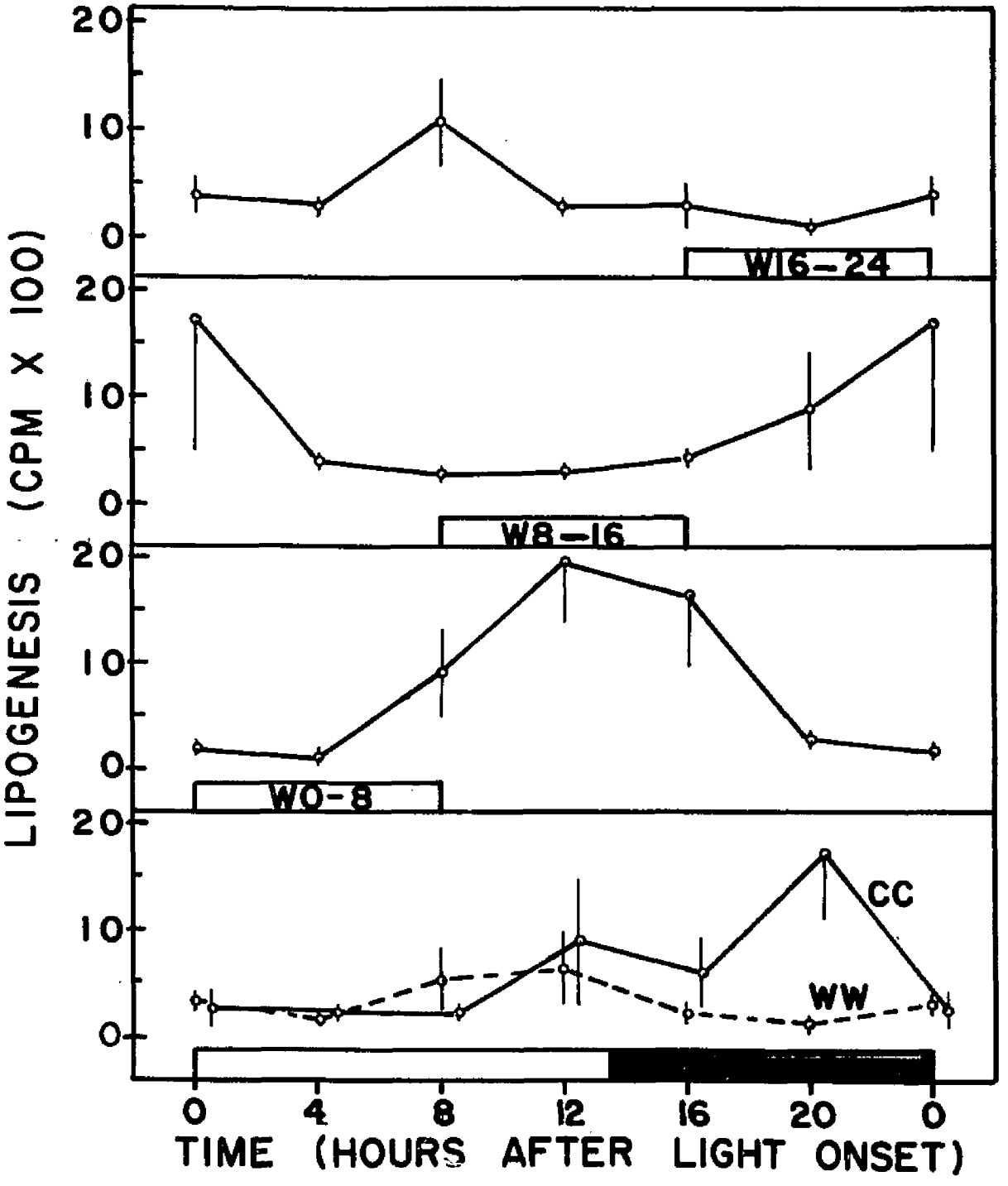

LIPOGENESIS (CPM X 100)
20
10
0
W16–24
W8–16
W0–8
CC
WW
0
4
8
12
16
20
0
TIME (HOURS AFTER LIGHT ONSET)

Figure 6. Time of day differences in fat body lipogenesis (^{3}H-acetate incorporation into lizard fat body lipid) in December. Green anoles were held on LD 10.5:13.5 and on one of five temperature regimes (see Figure 1 for explanation). Groups of animals were sampled once every 4 h for 24 h. Means are counts per minute ^{3}H-acetate incorporation into lipids of paired fat bodies.

Counts per minute were highest 4 h after heat onset in the thermoperiod groups ($P < .05$, Student's t test).

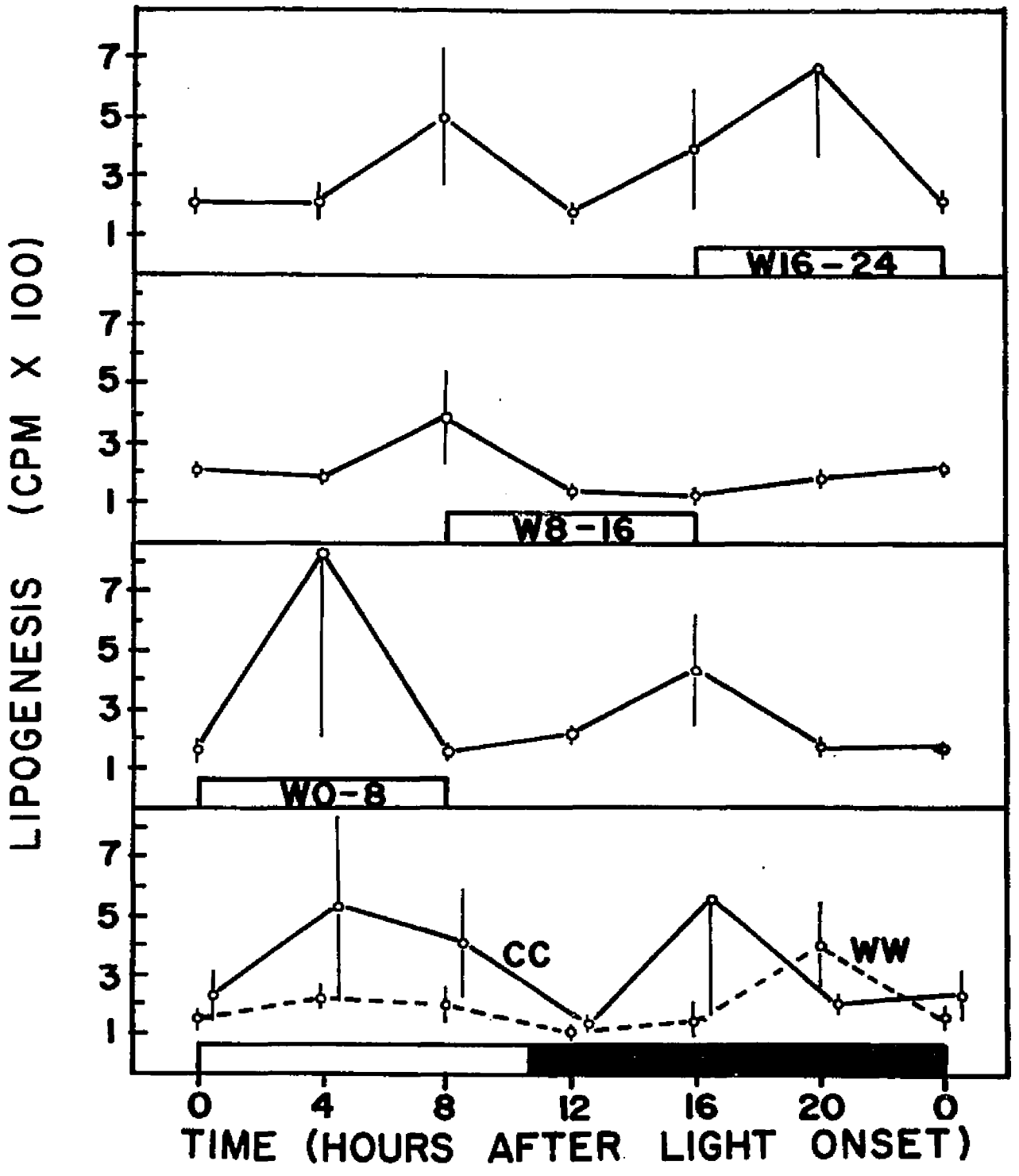
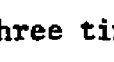
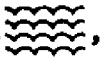
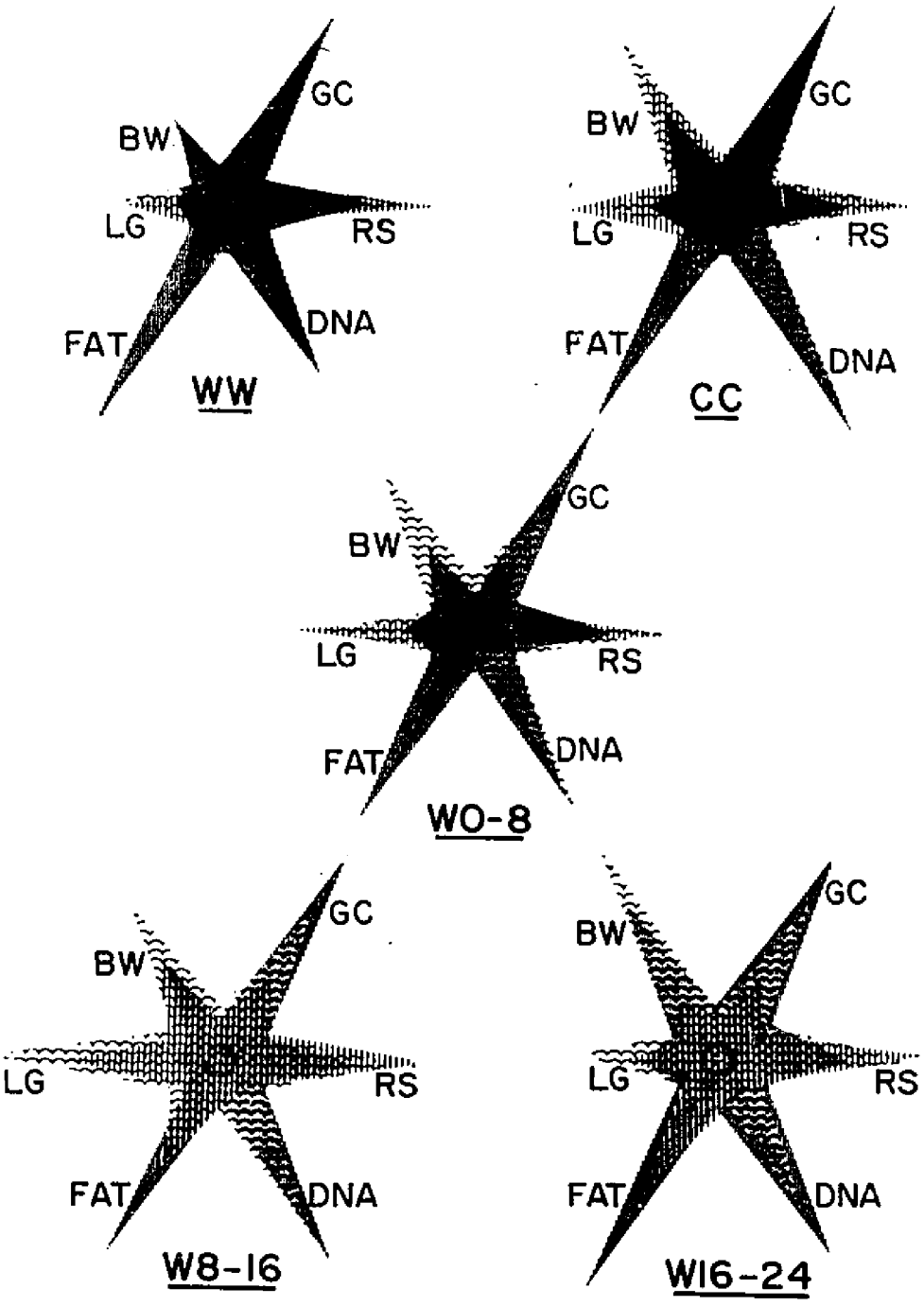

LIPOGENESIS (CPM X 100)
7
5
3
1
W16-24
W8-16
W0-8
CC
WW
0
4
8
12
16
20
0
TIME (HOURS AFTER LIGHT ONSET)

Figure 7. Pictorial summary of the effects of five temperature treatments (WW, CC, W0-8, W8-16, W16-24; see Figure 1 for explanation) on six variables at three times of year (April  , August  , and December ) in Anolis carolinensis. The abbreviations for the variables are: GC = number of germ cell rows, RS = reproductive stage, DNA = amount of DNA replication in testis, FAT = fat body wet weight, LG = lipogenesis in fat bodies, BW = change in body weight. Each point of the star represents a single variable, the length of the point from the center of the star represents the mean of the variable for each time of year.


GC
BW
LG
RS
FAT
DNA
WW
GC
BW
LG
RS
FAT
DNA
CC
GC
BW
LG
RS
FAT
DNA
WO-8
GC
BW
LG
RS
FAT
DNA
W8-16
GC
BW
LG
RS
FAT
DNA
W16-24

LITERATURE CITED

Bünning, E. 1973. The physiological clock. The English Universities Press, Ltd., London.

Burns, E.R., L.E. Scheving, J.E. Pauly, and T.-H. Tsui. 1976. Effect of altered lighting regimens, time-limited feeding, and presence of Ehrlich Ascites carcinoma on the circadian rhythm in DNA synthesis of mouse spleen. Can. Res. 36:1538-1544.

Burton, K. 1955. A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. Biochem. J. 62:315-323.

Clark, D.R., Jr., and J.C. Kroll. 1974. Thermal ecology of anoline lizards: Temperate versus tropical strategies. Southwest. Nat. 19:9-19.

Claussen, D.L. 1967. Studies of water loss in two species of lizards. Comp. Biochem. Physiol. 20:115-130.

Cloudsley-Thompson, J.L. 1960. Adaptive functions of circadian rhythms. Cold Spring Harbor Symp. Quant. Biol. 25:345-355.

Colquhoun, W.P. (ed.) 1971. Biological rhythms and human performance. Academic Press, London.

Cuellar, H.S. 1973. Effect of adiposectomy and feeding level on FSH-induced testicular growth in male lizards Anolis carolinensis (Reptilia : Iguanidae). J. Exp. Zool. 185:65-72.

Derickson, W.K. 1976. Lipid storage and utilization in reptiles. Am. Zool. 16:711-723.

Dessauer, H.C. 1953. Hibernation of the lizard, Anolis carolinensis. Proc. Soc. Exp. Biol. Med. 82:351-353.

Dessauer, H.C. 1955. Seasonal changes in the gross organ composition of the lizard, Anolis carolinensis. J. Exp. Zool. 128:1-12.

Dische, Z. 1955. Color reactions of nucleic acid components. In The nucleic acids. (E. Chargaff and J.N. Davidson, eds.). Vol. I, pp. 285-305. Academic Press Inc., New York.

Folch, J., M. Lees, and G.H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipides from animal tissues. J. Biol. Chem. 226:497-509.

Fox, W. 1958. Sexual cycle of the male lizard, Anolis carolinensis. Copeia 1958:22-29

Fox, W. and H.C. Dessauer. 1957. Photoperiodic stimulation of appetite and growth in the male lizard, Anolis carolinensis. J. Exp. Zool. 134:557-575.

Fox, W. and H.C. Dessauer. 1958. Response of the male reproductive system of lizards (Anolis carolinensis) to unnatural day-lengths in different seasons. Biol. Bull. 115:421-439.

Fuller, R.W. 1970. Daily variation in liver tryptophan, tryptophan pyrrolase, and tyrosine transaminase in rats fed ad libitum or single daily meals. Proc. Soc. Exp. Biol. Med. 133:620-622.

Gordon, R.E. 1956. The biology and biodemography of Anolis carolinensis. Ph.D. dissertation. Tulane Univ., New Orleans, Louisiana.

Gorman, G.C. and P. Licht. 1975. Differences between the reproductive cycles of sympatric Anolis lizards on Trinidad. Copeia 1975: 332-337.

Guraya, S.S. 1971. Histochemical observations on the lizard spermiogenesis. Acta anat. 79:270-279.

Hahn, W.E. and D.W. Tinkle. 1965. Fat-body cycling and experimental evidence for its adaptive significance to ovarian follicle development in the lizard Uta stansburiana. J. Exp. Zool. 158: 79-86.

Hamlett, G.W.D. 1952. Notes on breeding and reproduction in the lizard Anolis carolinensis. Copeia 1952:183-185.

Hoffmann, K. 1969a. Die relative Wirksamkeit von Zeitgeben. Oecologia 3:184-206.

Hoffmann, K. 1969b. Zum Einfluss der Zeitgeberstarke auf die Phasenlage der synchronisierten circadianen Periodik. Z. vergl. Physiol. 62:93-110.

Horseman, N.D., A.H. Meier, and D.D. Culley, Jr. 1976. Daily variations in the effects of disturbance on growth, fattening, and metamorphosis on the bullfrog (Rana catesbeiana) tadpole. J. Exp. Zool. 198:353-358.

King, F.W. 1966. Competition between two south Florida lizards of the genus Anolis. Ph.D. dissertation. Univ. Miami. Florida.

Kosh, R.J. and V.H. Hutchison. 1972. Thermal tolerance of parietal-ectomized Anolis carolinensis acclimatized at different temperature and photoperiods. Herpetologica 28:183-191.

Licht, P. 1964. The relation between thermoregulation and physiological adjustments to temperature in lizards. Ph.D. dissertation. Univ. Michigan.

Licht, P. 1965. Relation between preferred body temperatures and testicular heat sensitivity in lizards. Copeia 1964:428-436.

Licht, P. 1966. Reproduction in lizards: Influence of temperature on photoperiodism in testicular recrudescence. Science 154:1668-1670.

Licht, P. 1967a. Environmental control of annual testicular cycles in the lizard Anolis carolinensis. I. Interaction of light and temperature in the initiation of testicular recrudescence. J. Exp. Zool. 165:505-516.

Licht, P. 1967b. Environmental control of annual testicular cycles in the lizard Anolis carolinensis. II. Seasonal variations in the effects of photoperiod and temperature on testicular recrudescence. J. Exp. Zool. 166:243-254.

Licht, P. 1968. Response of the thermal preferendum and heat resistance to thermal acclimation under different photoperiods in the lizard Anolis carolinensis. Am. Midl. Nat. 79:149-158.

Licht, P. 1969a. Environmental control of annual testicular cycles in the lizard Anolis carolinensis. III. Temperature thresholds for photoperiodism. J. Exp. Zool. 172:311-322.

Licht, P. 1969b. Illuminance threshold and spectral sensitivity of photo-sexual responses in the male lizard, Anolis carolinensis. Comp. Biochem. Physiol. 30:233-246.

Licht, P. 1971a. Regulation of the annual testis cycle by photoperiod and temperature in the lizard Anolis carolinensis. Ecology 52: 240-252.

Licht, P. 1971b. Response of the male reproductive system to interrupted-night photoperiods in the lizard Anolis carolinensis. Z. vergl. Physiol. 73:274-284.

Licht, P. 1972. Problems in experimentation on timing mechanisms for annual physiological cycles in reptiles. In Hibernation and hypothermia, perspectives and challenges. (F.E. South, J.P. Hannon, J.R. Willis, E.T. Pengelley, and N.R. Alpert, eds.). pp. 681-711. Elsevier Pub. Co., Amsterdam.

Licht, P. and S.L. Basu. 1967. Influence of temperature on lizard testes. Nature 213:672-674.

Licht, P. and G.C. Gorman. 1970. Reproductive and fat cycles in Caribbean Anolis lizards. Univ. Calif. Publ. Zool. 95:1-62.

Licht, P. and G.C. Gorman. 1975. Altitudinal effects on the seasonal testis cycles of tropical Anolis lizards. Copeia 1975:496-504.

Licht, P. and R.E. Jones. 1967. Effects of exogenous prolactin on reproduction and growth in adult males of the lizard Anolis carolinensis. Gen. Comp. Endocrinol. 8:228-244.

Licht, P. and A.K. Pearson. 1969. Effects of adenohypophysectomy on testicular function in the lizard Anolis carolinensis. Biol. Reprod. 1:107-119.

Lima-de-Faria, A. and K. Borum. 1962. The period of DNA synthesis prior to meiosis in the mouse. J. Cell Biol. 14:381-388.

Luce, G.G. 1970. Biological rhythms in psychiatry and medicine. U.S. Publ. Health Serv. Publ. 2088:1-183.

Mayhew, W.W. 1965. Hibernation in the horned lizard Phrynosoma m'calli. Comp. Biochem. Physiol. 16:103-119.

Meier, A.H. 1975. Chronoendocrinology of vertebrates. In Hormonal correlates of behavior (B.E. Eleftheriou and R.L. Sprott, eds.). Vol. II. pp. 469-549. Plenum Press, New York.

Meier, A.H. 1977. Daily rhythms of lipogenesis in fat and lean white-throated sparrows, Zonotrichia albicollis. Am. J. Physiol. 232:193-196.

Meier, A.H. and J.T. Burns. 1976. Circadian hormone rhythms in lipid regulation. Am. Zool. 16:649-659.

Meier, A.H., T.N. Trobec, H.G. Haymaker, R. MacGregor, III, and A.C. Russo. 1973. Daily variations in the effects of handling on fat storage and testicular weights in several vertebrates. J. Exp. Zool. 184:281-288.

Michael, E.D. 1972. Growth rate in Anolis carolinensis. Copeia 1972:575-577.

Monesi, V. 1962. Autoradiographic study of DNA synthesis and the cell cycle in spermatogonia and spermatocytes of mouse testis using tritiated thymidine. J. Cell Biol. 14:1-18.

Noeske, T.A. 1974. The effects of photoperiod and thermoperiod on the reproductive cycle of the male green anole, Anolis carolinensis. Master's thesis. Louisiana State Univ., Baton Rouge.

Noeske, T.A. and A.H. Meier. 1977. Photoperiodic and thermoperiodic interaction affecting fat stores and reproductive indexes in the male green anole, Anolis carolinensis. J. Exp. Zool. 202:97-102.

Oliver, J.A. 1955. The natural history of North American amphibians and reptiles. D. Van Nostrand Company, Inc., Princeton, New Jersey.

Palmer, J.D. 1976. An introduction to biological rhythms. Academic Press, New York.

Parish, J.H. 1972. Principles and practice of experiments with nucleic acids. Longman Group Limited, London.

Pearson, A.K., H.W. Tsui, and P. Licht. 1976. Effect of temperature on spermatogenesis, on the production and action of androgens, and on the ultrastructure of gonadotropic cells in the lizard Anolis carolinensis. J. Exp. Zool. 195:291-304.

Pengelley, E.T. (ed.) 1974. Circannual clocks; Annual biological rhythms. Academic Press, Inc., New York.

Prosser, C.L. and F.A. Brown, Jr. 1961. Comparative animal physiology. 2nd edition. W.B. Saunders Company, Philadelphia.

Reynolds, W.W. 1977. Circadian rhythms in the goldfish Carassius auratus L.: Preliminary observations and possible implications. Rev. Can. Biol. 36:355-356.

Rose, F.L. 1967. Seasonal changes in lipid levels of the salamander Amphiuma means. Copeia 1967:662-666.

Ruby, J.R., L.E. Scheving, S.B. Gray, and K. White. 1973. Circadian rhythm of nuclear DNA in adult rat liver. Exp. Cell Res. 76:136-142.

Schneider, W.C. 1945. Phosphorus compounds in animal tissues. I. Extraction and estimation of desoxypentose nucleic acid and of pentose nucleic acid. J. Biol. Chem. 161:293-303.

Shatkin, A.J. 1969. Colorimetric reactions for DNA, RNA, and protein determinations. In Fundamental techniques in virology (K. Habel and N.P. Salzman, eds.). pp. 231-237. Academic Press, New York.

Spieler, R.E. 1977. Diel and seasonal changes in response to stimuli: A plague and a promise for mariculture. Proc. World Maricult. Soc. 8:865-882.

Spieler, R.E., T.A. Noeske, V. deVlaming, and A.H. Meier. 1977a. Effects of thermocycles on body weight gain and gonadal growth in the goldfish, Carassius auratus. Trans. Am. Fish. Soc. 106: 440-444.

Spieler, R.E., A.H. Meier, and T.A. Noeske. 1977b. Timing of a single daily meal affects daily serum prolactin rhythm in gulf killifish, Fundulus grandis. Life Sci. 22:255-258.

Spieler, R.E., A.H. Meier, and T.A. Noeske. 1978. Temperature-induced phase shift of daily rhythm of serum prolactin in gulf killifish. Nature 271:469-470.

Trobec, T.N. 1974. Circadian mechanisms in the hormonal control of annual cycles of fat storage and reproduction in the lizard, Anolis carolinensis. Ph.D. dissertation. Louisiana State Univ., Baton Rouge.

Webb, J.M. and H.V. Lindstrom. 1965. Acid solubilization of animal tissue nucleic acids as related to their extraction and estimation. Arch. Biochem. Biophys. 112:273-281.

Windell, J.T. and J.A. Sarokon. 1976. Rate of gastric evacuation in the lizard, Anolis carolinensis (Sauria : Iguanidae). Herpetologica 32:18-23.

Wurtman, R.J., C.M. Rose, C. Chou, and F.F. Larin. 1968. Daily rhythms in the concentrations of various amino acids in human plasma. New Engl. J. Med. 279:171-175.

APPENDIX

In the majority of physiological studies, only one reproductive variable is used as an indicator of reproductive state. Gonad wet weight and gonadosomatic index (GSI = gonad wet weight (g) / final body weight (g) x 100) are the most common (and easiest to measure) of these variables. However, recent literature involving histological studies in ectotherms has reported that these variables may be misleading (Licht, 1967a). Because I am interested in reproduction in ectotherms and in obtaining as clear a picture as possible of the reproductive state, I measured several variables mentioned by other researchers as good indicators of the reproductive state of the male green anole. Besides gonad wet weight and GSI, these included the number of germ cell rows in the seminiferous tubules, the diameter of the seminiferous tubules, the most abundant and the most advanced germ cell types in the seminiferous tubules, the number of germ cell rows x the diameter of the seminiferous tubules (Fox, 1958), and reproductive stage (Fox-Licht stage, see Table I). The rate of DNA replication (as counts per minute of ^{3}H-thymidine incorporation in the testis) was also measured.

No single reproductive variable examined in this study was a reliable indicator of the reproductive state of the male green anole. Gonad wet weight gives good among-treatment and among-season comparisons. However, gonad weight does not determine whether a treatment is stimulatory or inhibitory, even with baseline data. A small gonad may be either regressing or recrudescing. The reproductive cycle and gonad weight of A. carolinensis are well documented (Dessauer,

1955; Fox, 1958). Thus, if an animal were sampled from the wild and the gonads weighed, the reproductive state would be known. In the natural reproductive cycle, there are regular correlations between histological observations and gonad weight. However, in laboratory experiments with abnormal photoperiod or temperature regimes, these correlations no longer hold. Histological examination is necessary to determine whether the testis is regressing or recrudescing. Thus, gonad weight in laboratory experiments should be qualified by histology.

The GSI has assets and liabilities similar to those of gonad weight. It is good for between-treatment comparisons, but should be qualified by histological evaluation. Also, the GSI incorporates possible variations in final body weight. In this study, the within-treatment variation of GSI was increased over that of gonad wet weights, particularly the thermoperiod groups. (For the same reason, fat body wet weight is a more reliable variable than fat body weight as a percent of body weight.)

Of the histological variables examined, the Fox-Licht reproductive stage is a good index, particularly for between-temperature treatment comparisons. The stage may indicate real differences between treatments that are not revealed by gonad weight. For example, in December, gonads of CC and WW animals were the same weight (Table XIII), but the stage was significantly different (Table XXV). In this case, the added observation of most abundant cell type is of interest. In WW, spermatids were the most abundant cell type; in CC spermatocytes were the most abundant. Reproductive stage emphasizes spermiation and

the most advanced, and in later stages, the most abundant germ cell type. However, it does not take into consideration the reproductive potential of the animal. In animals held in WW and long photoperiod for several months, the gonads are at a high reproductive stage, but the gonad weight and the number of germ cell rows decrease (Licht, 1966). This does not mean that gonad weight is a poor indicator of reproductive state. It means that the animal is held on an abnormal regime, and the replication of early germ cells is not in balance with spermiation. Thus, gonad weight and/or the number of germ cell rows should be compared with reproductive stage to give an estimate of reproductive potential of the animal.

Because both the weight and the histological variables are "static" variables (give no indication of rate of change), the rate of DNA replication (^{3}H-thymidine incorporation into the testis) may be another important consideration. For example, the August CC animals have large gonads with many germ cell rows (Tables XII, XVIII). However, the rate of DNA replication is extremely low - gonadal activity is practically stopped (Table XXXIII). In contrast, the WW gonad is almost involuted, and yet is more active (higher rate of DNA replication).

Although DNA synthesis in somatic tissues is often expressed as counts per minute / mg DNA, I consider this variable inappropriate for the gonads. The testis is not similar to somatic tissues in which all cells may acquire label. In the testis, with the short incubation time used, only spermatogonia and primary spermatocytes would be labelled. Only after many successive divisions (taking longer than 4 h) would label appear in the spermatids or spermatozoa. Thus,

a testis with a high rate of mitosis and meiosis may have a lower count per minute DNA / mg DNA because of a greater amount of unlabelled spermatozoa and spermatids than an involuted testis (3 germ cell rows) which is very slowly forming new cells.

In summary, several variables must be examined to picture adequately the reproductive state of the green anole. At best, three variables should be considered: reproductive potential (indicated by gonad wet weight, number of germ cell rows), reproductive stage, and rate of DNA replication.

VITA

Teresa A. Noeske was born 14 August 1951 in Freeport, Illinois. She received a Bachelor of Science degree in zoology in 1972 and a Master of Science degree in physiology in 1974 from Louisiana State University in Baton Rouge. Ms. Noeske entered the doctoral degree program in the Department of Zoology and Physiology of Louisiana State University in December, 1974. She has been employed as a research assistant at the Milwaukee Public Museum since 1975. She is also a candidate for the degree of Doctor of Philosophy at Louisiana State University.